PERIODICAL ROOM
RECEIVED
JUN 2 1916
UNIV. OF MICH.
LIBRARY

# THE JOURNAL
OF
# EXPERIMENTAL MEDICINE

EDITED BY
SIMON FLEXNER, M.D.

VOLUME XXIII, NO. 6 JUNE 1, 1916

PUBLISHED MONTHLY
AT 2419-21 GREENMOUNT AVE., BALTIMORE, MD.
BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Application has been made for entry as second-class matter at the Post Office at Baltimore, Md., under the Act of March 3, 1879

COPYRIGHT, 1916, BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

WAVERLY PRESS
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

# EXPERIMENTS ON THE DEVELOPMENT OF MALARIA PARASITES IN THREE AMERICAN SPECIES OF ANOPHELES.

By W. V. KING, Ph.D.

*(From the Bureau of Entomology, U. S. Department of Agriculture, Washington, D. C., and the Laboratories of Clinical Medicine of the School of Medicine of Tulane University, New Orleans.)*

Plates 98 to 105.

(Received for publication, March 20, 1916.)

The early results of experiments to determine the susceptibility of *Anopheles punctipennis* to infection with the parasites of tertian malaria have been recorded in two recent articles by the writer (1915–1916). In these it was shown that this species is an efficient host of *Plasmodium vivax*, and in the second article a foot-note was appended stating that it had also proven to be a host of *Plasmodium falciparum*, the parasite of estivo-autumnal malaria.

In the present paper are given further details of this series of experiments in which the susceptibility of *Anopheles crucians*, as well as of *Anopheles punctipennis*, has been established. The information acquired upon the relative susceptibility of these two species together with *Anopheles quadrimaculatus* is also included.

A study of the literature applying to this subject has revealed the fact that an extremely small amount of experimental work has actually been done with the species of *Anopheles* occurring in the United States. The experiments with *Anopheles punctipennis* were contradictory and inconclusive, and, as stated in the previous articles, the impression had become current that it was not a factor in the transmission of malaria. The proof of its efficiency as a host of malaria parasites does not, of course, establish the degree of its importance in the transmission of malaria. This depends as well upon its food habits in relation to man, its prevalence, and distribution.

In regard to *A. crucians*, nothing has been found in the early literature which establishes with certainty the proof of its susceptibility, although it has been included among the efficient hosts by several authors, notably Howard, Dyar, and Knab (1912), and Knab (1913). They refer to the experiments reported by Beyer, Pothier, Couret, and Lemann (1902), as Dupree (1905) undoubtedly does also in accrediting the proof to Couret and Beyer. The statements are evidently based upon a misinterpretation of the report.

The results of the experiments by Beyer and his associates may be summarized as follows: With *A. crucians*, no infections were obtained with tertian parasites in two specimens or with quartan parasites in three specimens, while none were tested with estivo-autumnal malaria. With *A. quadrimaculatus*, one specimen of three became infected with tertian malaria and two specimens of five with quartan. The five fed on blood containing estivo-autumnal parasites were negative. Their incrimination of *A. crucians* as a host of estivo-autumnal malaria was based on epidemiologic considerations.

Mitchell (1907) made the statement that Dupree had shown her the parasites in the salivary glands of three species (*A. quadrimaculatus*, *A. crucians*, and *A. punctipennis*). Dupree himself, however, made no reference to his having obtained infected specimens of *quadrimaculatus* or *crucians*.

Recently Mitzmain (1916) obtained negative results with 219 *A. punctipennis* dissected from 3 to 38 days after multiple bites on individuals whose blood contained varying numbers of estivo-autumnal crescents. One specimen of three *A. crucians* fed on one of the gamete carriers showed an infection.

The susceptibility of *A. quadrimaculatus* was established by the work of Thayer (1900), who obtained positive results with this species for both estivo-autumnal and tertian malaria—(one infected specimen with the former and two with the latter). Woldert (1901) conducted nine experiments with *A. quadrimaculatus*. The first experiment with tertian malaria failed. Fourteen specimens dissected in the following six experiments with tertian were all negative. In the last two, with estivo-autumnal malaria, two positive specimens were found among the seven dissected. Berkeley (1901) succeeded after repeated experiments in inoculating *A. quadrimaculatus* with the tertian parasite. Hirshberg (1904) obtained eight infected *A. quadrimaculatus* in a series of forty-eight fed on five estivo-autumnal gamete carriers. Beyer, Pothier, Couret, and Lemann reported infections in this species after the ingestion of the parasites of tertian and quartan malaria.

A fourth species of *Anopheles* (*A. pseudopunctipennis*), reported from the Southern United States, was found positive for the parasite of estivo-autumnal malaria in experiments by Darling (1910) in the Panama Canal Zone. Out of thirty-one specimens dissected, four were found to be infected. From the small number infected under the most favorable artificial conditions, and because of the fact that relatively few of this species are taken in dwellings, Darling concludes that *pseudopunctipennis* is only slightly concerned in the transmission of malaria.

The importance of the accurate determination of the exact relation of each species of *Anopheles* to malaria transmission has been emphasized by the results of a number of noteworthy investigations, which have shown that the greatest variation in susceptibility exists among the different species of this genus. Some species are entirely immune, while certain species manifest a difference in susceptibility to the different species of malaria plasmodia. Walker and Barber (1914) have given an excellent discussion of these phases of the subject in the introduction to their paper on "Malaria in the Philippine Islands."

## EXPERIMENTAL.

The experiments reported below were conducted in New Orleans during the months of November and December, 1915, and January, 1916. The adult mosquitoes of the two species, *Anopheles quadrimaculatus* and *Anopheles punctipennis* were bred from larval material supplied by Mr. D. L. Van Dine, of the Bureau of Entomology, from Mound, La. Since no bred material of *Anopheles crucians* was available, specimens collected in the open were used. A flight of this species into the city occurred at this time and upon examination it was found that only a very small proportion of them had had blood meals. The specimens used for infecting purposes were selected from the ones in which no distention of the abdomen with blood or developed ova could be detected.

The experimental mosquitoes were given but one meal of infected blood. They were fed individually, not in lots as is usually done, so that the fact of their having fed was thus made certain in each case. Before the blood meal and afterwards, the diet consisted of raisins and water. Between the times of the blood meal and dissection, each specimen was kept in a separate container.

In Table I is given a part of the data obtained from the examinations of females of *Anopheles punctipennis* after they had fed on estivo-autumnal gamete carriers. The examinations were made at intervals ranging from 7 to 46 days. In all, twenty-two specimens were fed on two patients. Two specimens were not examined. On one of the patients four feedings were made on different days. Case 514 showed a medium number of gametes in the blood, but since no other species besides *punctipennis* were fed on this case, a satisfactory explanation of the negative results cannot be made.

TABLE I.

*Results of Experiments with Anopheles punctipennis and Estivo-Autumnal Parasites.*

| Specimen No. | Date of feeding. | Date of dissection. | Interval. | Result of examination. | | Case No. |
|---|---|---|---|---|---|---|
| | | | | Midgut. | Salivary glands. | |
| | *1915* | *1915* | *days* | | | |
| 602.1 | Nov. 13 | Dec. 30 | 47 | − | + | 511 |
| 602.2 | " 13 | " 15 | 32 | − | − | 511 |
| 617.1 | " 13 | Nov. 25 | 12 | − | Not examined. | 511 |
| 617.2 | " 13 | — | | | | 511 |
| 617.3 | " 13 | Dec. 6 | 23 | − | − | 511 |
| 618.1 | " 13 | " 29 | 46 | − | − | 511 |
| 618.2 | " 13 | " 27 | 44 | − | − | 511 |
| 618.3 | " 13 | " 27 | 44 | − | − | 511 |
| 612.6 | " 22 | " 21 | 29 | − | − | 511.5 |
| 626.1 | " 22 | " 21 | 29 | + | + | 511.5 |
| 617.4 | " 23 | " 14 | 21 | + | + | 511.6 |
| 618.5 | " 23 | Nov. 30 | 7 | − | − | 511.6 |
| 620.1 | " 23 | Dec. 15 | 22 | + | − | 511.6 |
| | | *1916* | | | | |
| 601.1 | Dec. 23 | Jan. 17 | 25 | − | − | 514 |
| 601.2 | " 23 | " 14 | 22 | − | − | 514 |
| 601.3 | " 23 | — | | | | 514 |
| 601.4 | " 23 | " 17 | 25 | − | − | 514 |
| 604.1 | " 23 | " 15 | 23 | − | − | 514 |
| 606.1 | " 23 | " 15 | 23 | − | − | 514 |
| 607.1 | " 23 | " 14 | 22 | − | − | 514 |
| 607.2 | " 23 | " 14 | 22 | − | − | 514 |
| 610.1 | " 23 | " 17 | 25 | − | − | 514 |

Total No. dissected..... 20
" " infected..... 4
Per cent " ..... 20
" " " of those fed on Case 511 only..... 33

In Specimen 602.1, the salivary glands contained only a very few sporozoites. In No. 626.1, one oocyst and one empty capsule were found upon the stomach, and the salivary glands contained large numbers of sporozoites in all the lobes of both sets. In No. 617.4 (Figs. 7, 8, and 14), one oocyst and one empty capsule were present on the stomach. The oocyst showed well developed sporoblasts. The salivary glands of this specimen were heavily infected with

sporozoites. In No. 620.1, eight oocysts were counted on the stomach, five of which were placed toward the anterior end of the enlargement of the midgut. Two of the oocysts were in the last stage of development before the release of the sporozoites, and four were large but without visible sporoblasts. The measurement of the latter gave: 47 by 48, 31 by 37, 35 by 40, and 48 by 55 microns. The other two were very small—not over 20 microns in diameter.

In Table II are shown the results of the examination of females of *Anopheles punctipennis* after feeding on tertian gamete carriers. Further details of this series have been given in a previous article (King, 1916).

TABLE II.

*Results of Experiments with Anopheles punctipennis and Tertian Parasites.*

| Specimen No. | Date of feeding. | Date of dissection. | Interval. | Result of examination. | | Case No. |
|---|---|---|---|---|---|---|
| | | | | Midgut. | Salivary glands. | |
| | *1915* | *1915* | *days* | | | |
| 612.1* | Nov. 6 | Nov. 24 | 18 | + | − | 509 |
| 612.2 | " 6 | " 15 | 9 | + | Not examined. | 509 |
| 612.3 | " 6 | Dec. 1 | 25 | − | − | 509 |
| 614.1† | " 6 | Nov. 26 | 20 | + | + | 509 |
| 614.2 | " 6 | " 13 | 7 | + | Not examined. | 509 |
| 614.3 | " 6 | " 13 | 7 | + | " " | 509 |
| 612.5‡ | " 12 | Dec. 2 | 20 | + | + | 510 |

Total No. dissected..... 7
" " infected..... 6
Per cent " ..... 85

* Figs. 2, 3, and 4. † Figs. 9 and 11. ‡ Fig. 10.

Table III shows the results obtained with specimens of *Anopheles crucians* fed upon an estivo-autumnal gamete carrier. A total of thirty-five females were fed during a period of 5 days, but this species lived poorly in captivity, and sixteen which died soon after the blood meal were unsuitable for examination. The fact that they were "wild" mosquitoes and not bred, as were the other two

species, may account for this high mortality. Seven other specimens which were used in another experiment could not be included in the tabulation.

TABLE III.

*Results of Experiments with Anopheles crucians and Estivo-Autumnal Parasites.*

| Specimen No. | Date of feeding. | Date of dissection. | Interval. | Result of examination. | | Case No. |
|---|---|---|---|---|---|---|
| | | | | Midgut. | Salivary glands. | |
| | *1915* | *1915* | *days* | | | |
| 703.1 | Nov. 23 | Dec. 7 | 14 | − | − | 511.6 |
| 703.2 | " 23 | " 16 | 23 | − | − | 511.6 |
| 703.4 | " 23 | " 3 | 10 | − | Not examined. | 511.6 |
| 703.6 | " 23 | " 13 | 20 | + | − | 511.6 |
| 703.8 | " 23 | " 16 | 23 | − | + | 511.6 |
| 703.9 | " 23 | " 20 | 27 | − | + | 511.6 |
| 703.12 | " 23 | " 9 | 16 | + | Not examined. | 511.6 |
| 705.8 | " 24 | " 24 | 30 | − | + | 511.7 |
| 709.1 | " 27 | " 27 | 30 | + | + (?) | 511.9 |
| 710.2 | " 27 | " 21 | 24 | + | − | 511.9 |
| 710.3 | " 27 | " 26 | 29 | + | + (?) | 511.9 |
| 710.4 | " 27 | " 27 | 30 | + | − | 511.9 |

Total No. dissected..... 12
" " infected..... 9
Per cent " ..... 75

Specimen 703.6 (Figs. 1, 5, and 6), had a very large number of oocysts on the stomach; the number was estimated at 75 after 39 had been counted. These ranged in size from 24 to 50 microns in diameter, the majority probably between 40 and 50. No. 703.8 had a medium infection of the center lobes of the salivary glands (compare Fig. 12). A heavy infection of all the lobes of the glands existed in No. 703.9. Five oocysts were present on the stomach of No. 703.12. Two of these measured 34 by 37 microns. In No. 705.8 a small number of sporozoites were found in the salivary glands. In No. 709.1, one large oocyst, 50 by 60 microns, was present on the stomach. The condition of the salivary glands was such that the presence of sporozoites could not be definitely ascertained. One oocyst measuring 45 microns in diameter was found on the stomach

of No. 710.2. In No. 710.3, one oocyst was present on the stomach and the glands seemed to contain sporozoites, but their condition made the diagnosis uncertain. In No. 710.4, one oocyst was seen on the stomach.

Table IV shows the infections occurring in specimens of *Anopheles quadrimaculatus* with tertian parasites, and Table V with estivo-autumnal parasites.

TABLE IV.

*Results of Experiments with Anopheles quadrimaculatus and Tertian Parasites.*

| Specimen No. | Date of feeding. | Date of dissection. | Interval. | Result of examination. | | Case No. |
|---|---|---|---|---|---|---|
| | | | | Midgut. | Salivary glands. | |
| | *1915* | *1915* | *days* | | | |
| 349.2 | Nov. 2 | Nov. 11 | 9 | − | Not examined. | 507 |
| 351.3 | " 2 | " 6 | 4 | − | " " | 507 |
| 352.7 | " 2 | " 10 | 8 | − | " " | 507 |
| 352.8 | " 2 | " 9 | 7 | + | " " | 507 |
| 352.9 | " 2 | " 12 | 10 | + | " " | 507 |
| 365.1* | " 6 | Dec. 7 | 31 | + | + | 509 |
| 365.2 | " 6 | − | | | | 509 |
| 372.1 | " 6 | − | | | | |
| 373.1 | " 6 | Nov. 16 | 10 | + | − | 509 |
| 373.2 | " 6 | " 19 | 13 | + | Not examined. | 509 |
| 373.3 | " 6 | − | | | | |
| 347.6 | " 12 | Dec. 9 | 27 | − | + | 510 |
| 365.5† | " 12 | " 9 | 27 | − | + | 510 |
| 365.6 | " 12 | " 13 | 31 | − | − | 510 |
| 371.1 | " 12 | − | | | | |
| 373.4 | " 12 | − | | | | |
| 373.5‡ | " 12 | Nov. 30 | 18 | − | + | 510 |
| 374.1 | " 12 | − | | | | |

Total No. dissected..... 12
" " infected..... 8
Per cent " ..... 66

* Figs. 16 and 17. † Fig. 13. ‡ Fig. 15.

TABLE V.

*Results of Experiments with Anopheles quadrimaculatus and Estivo-Autumnal Parasites.*

| Specimen No. | Date of feeding. | Date of dissection. | Interval. | Result of examination. | | Case No. |
|---|---|---|---|---|---|---|
| | | | | Midgut. | Salivary glands. | |
| | *1915* | *1915* | *days* | | | |
| 349.1 | Nov. 2 | Nov. 13 | 11 | — | Not examined. | 506 |
| 354.1 | " 2 | " 10 | 8 | — | " " | 506 |
| 358.1 | " 2 | " 15 | 13 | — | " " | 506 |
| 358.2 | " 2 | " 15 | 13 | — | " " | 506 |
| 359.1 | " 2 | — | | | | 506 |
| 359.2 | " 2 | " 8 | 6 | — | " " | 506 |
| 359.3 | " 2 | " 8 | 6 | — | " " | 506 |
| 347.7 | " 13 | Dec. 6 | 23 | — | — | 511 |
| 350.1 | " 13 | " 1 | 18 | — | — | 511 |
| 350.2 | " 13 | — | | | | |
| 350.3 | " 13 | " 29 | 46 | — | — | 511 |
| 350.4 | " 13 | " 18 | 35 | — | + | 511 |
| 365.7 | " 13 | " 28 | 45 | + | — | 511 |
| 371.2 | " 13 | Nov. 20 | 7 | — | Not examined. | 511 |
| 380.1 | " 22 | — | | | — | 511.5 |
| 386.1 | " 23 | Dec. 14 | 21 | — | — | 511.6 |
| 390.1 | " 23 | " 14 | 21 | + | — | 511.6 |
| 381.1 | " 24 | " 21 | 27 | — | — | 511.7 |
| 803.1 | " 24 | " 15 | 21 | — | — | 511.7 |
| 381.2 | " 26 | — | | | | 511.8 |
| 394.1 | " 27 | " 22 | 25 | — | — | 511.9 |
| 396.1 | " 29 | " 13 | 14 | — | — | 511.10 |
| 396.2 | " 29 | " 22 | 23 | — | — | 511.10 |

Total No. dissected..... 19
" " infected..... 3
Per cent " ..... 15
" " " of those fed on Case 511..... 23

Since no infections resulted from Case 506, the percentage from Case 511 only is shown.

The total number of mosquitoes of the three species examined is too small to permit of very satisfactory comparisons, but the indications of relative susceptibility as shown in Tables VI and VII are of interest. In these only those specimens which were fed on the same gamete carriers have been included. Since the females of

*Anopheles crucians* were not bred, while those of the other two species were, the results are perhaps not strictly comparable.

TABLE VI.

*Comparative Results with Plasmodium vivax.*

| | *A. punctipennis.* | *A. quadrimaculatus.* |
|---|---|---|
| Total No. dissected | 7 | 7 |
| " " infected | 6 | 6 |
| Per cent " | 85 | 85 |

TABLE VII.

*Comparative Results with Plasmodium falciparum.*

| | *A. punctipennis.* | *A. quadrimaculatus.* | *A. crucians.* |
|---|---|---|---|
| Total No. dissected | 12 | 13 | 12 |
| " " infected | 4 | 3 | 9 |
| Per cent " | 33 | 23 | 75 |

In Table VIII are shown the proportion of gametes to leukocytes in the blood upon which the mosquitoes fed. Case 511 was employed for feeding purposes on several different days extending over a period of 2 weeks. As may be seen from the earlier counts, the numbers of gametes were extremely high.

TABLE VIII.

*Comparative Counts of Leukocytes and Gametes Made from Stained Blood Smears Taken at the Time of the Feeding of the Mosquitoes.*

| Case No. | Species of *Plasmodium.* | No. of leukocytes counted. | Gametes. | Gametes per 100 leukocytes. |
|---|---|---|---|---|
| 509 | *P. vivax.* | 131 | 19 | 14 |
| 511 | " *falciparum.* | 57 | 300 | 526 |
| 511.5 | " " | 100 | 143 | 143 |
| 511.6 | " " | 200 | 187 | 93 |
| 511.7 | " " | 125 | 171 | 136 |
| 511.8 | " " | 350 | 106 | 30 |
| 511.9 | " " | 350 | 128 | 36 |
| 511.10 | " " | 300 | 56 | 18 |
| 514 | " " | 281 | 20 | 7 |

The mosquitoes used in the experiments were kept in a darkened, screened box in the laboratory. Temperature and humidity records for the entire period were kept by means of Friez recording instruments placed beside the box. The temperature of this room was usually higher and fluctuated less than the outdoor temperature, and during part of the time the room was artificially heated during the day. The temperature in the laboratory rarely fell below 60°F. On Dec. 28, however, the minimum was 51°, and on the 29th, 49°.

TABLE IX.

*Weekly Mean Temperature and Per Cent Humidity (Relative) from November 1, 1915, to January 16, 1916.*

| Week. | Mean temperature. | Average relative humidity. |
|---|---|---|
| | °F. | *per cent* |
| Nov. 1–7 | 76.7 | 60.3 |
| " 8–14 | 79.7 | 65.5 |
| " 15–21 | 71.6 | 48.7 |
| " 22–28 | 73.5 | 57.6 |
| " 29–Dec. 5 | 68.5 | 48.8 |
| Dec. 6–12 | 72 | 64.0 |
| " 13–19 | 68.7 | 62.0 |
| " 20–26 | 65.4 | 54.2 |
| " 27–Jan. 2 | 64.7 | 72.2 |
| Jan. 3–9 | 75 | 64.4 |
| " 10–16 | 69.1 | 51.6 |

An explanation of the long developmental period of the parasites, as exhibited in these experiments, is undoubtedly found in the temperature conditions prevailing during the time. The length of the sexual cycle is usually given as from 9 to 12 days, but the exact relation of temperature to the period of development has not been carefully ascertained.

SUMMARY.

Since a knowledge of the susceptibility of any species of *Anopheles* to infection with malaria parasites is of great importance in determining its part in the transmission of malaria, the experiments reported here were undertaken, and included the three most prevalent species of this genus occurring in the United States. As a result of these experiments *Anopheles punctipennis* is shown to be an efficient

host of the organisms of tertian and estivo-autumnal malaria, *Anopheles crucians* of estivo-autumnal malaria, at least, and information has been obtained upon the relative susceptibility of these two species and *Anopheles quadrimaculatus*. The latter species has been known to be an efficient host since Thayer's experiments in 1900, and has been considered to be the principal species concerned in the transmission of malaria in the United States.

With *Anopheles punctipennis*, developmental forms of the exogenous or sporogenic cycle of *Plasmodium vivax* were demonstrated in six (85 per cent) of the seven mosquitoes dissected, and the development of *Plasmodium falciparum*, in four (20 per cent) of twenty specimens. These four infections, however, occurred in a series of thirteen specimens fed on one person, so that the percentage was actually 33.

With *Anopheles crucians*, oocysts or sporozoites or both oocysts and sporozoites of *Plasmodium falciparum* were found in nine (75 per cent) of the twelve specimens dissected. No tests were made with this species and *Plasmodium vivax*.

*Anopheles quadrimaculatus* was employed as a control species in the experiments and became infected in the following ratio: eight (66 per cent) of twelve specimens with *Plasmodium vivax*, and three (15 per cent) of nineteen specimens with *Plasmodium falciparum*.

In determining the relative susceptibility of the three species only those individuals which had fed upon the same gamete carriers are considered. The number of mosquitoes from which the percentages are computed is too small to make the results entirely conclusive, but the indications are that *Anopheles punctipennis* and *Anopheles quadrimaculatus* are equally susceptible to infection with *Plasmodium vivax*, 85 per cent of each species under the same conditions being positive. With *Plasmodium falciparum*, *Anopheles crucians* showed the highest percentage of infection (75 per cent), *Anopheles punctipennis* second (33 per cent), and *Anopheles quadrimaculatus* third (23 per cent).

The writer desires to acknowledge the cooperation and advice received from Dr. C. C. Bass and Dr. F. M. Johns, of the Laboratories of Clinical Medicine of the School of Medicine of Tulane University.

## BIBLIOGRAPHY.

Berkeley, W. N., Some Further Work on the Mosquito-Malaria Theory, with Special Reference to Conditions around New York, *Med. Rec.*, 1901, lix, 124–128.

Beyer, G. E., Pothier, O. L., Couret, M., and Lemann, I. I., Bionomics, Experimental Investigations with *Bacillus sanarelli* and Experimental Investigations with Malaria, *New Orleans Med. and Surg. Jour.*, 1901–02, liv, 419–480.

Darling, S. T., Studies in Relation to Malaria, *U. S. Government Printer*, Washington, 1910.

Dupree, W. H., The Mosquitoes of Louisiana and Their Pathogenic Possibilities with Remarks upon Their Extermination, *New Orleans Med. and Surg. Jour.*, 1905–06, lviii, 1–16.

Hirshberg, L. K., An *Anopheles* Mosquito Which Does Not Transmit Malaria. *Bull. Johns Hopkins Hosp.*, 1904, xv, 53–56.

Howard, L. O., Dyar, H. G., and Knab, F., The Mosquitoes of North and Central America and the West Indies, *Carnegie Institution of Washington*, 1912, No. 159, i, 202.

King, W. V., The rôle of *Anopheles punctipennis* Say in the Transmission of Malaria, *Science*, 1915, xlii, 873–874.

King, W. V., *Anopheles punctipennis*, a Host of Tertian Malaria, *Am. Jour. Trop. Dis. and Prev. Med.*, 1916, iii, 426–432.

Knab, F., The Species of *Anopheles* That Transmit Human Malaria, *Am. Jour. Trop. Dis. and Prev. Med.*, 1913, i, 33–43.

Mitchell, E. G., Mosquito Life; the Habits and Life Cycles of the Known Mosquitoes of the United States; Methods for Their Control; and Keys for Easy Identification of the Species in Their Various Stages, New York and London, 1907, 136.

Mitzmain, M., *Anopheles punctipennis* Say. Its Relation to the Transmission of Malaria.—Report of Experimental Data Relative to Subterranean Malaria Fever, *Public Health Reports*, 1916, xi, 301–307.

Thayer, W. S., On Recent Advances in Our Knowledge Concerning the Etiology of Malarial Fever, *Philadelphia Med. Jour.*, 1900, v, 1046–1048.

Walker, E. L., and Barber, M. A., Malaria in the Philippine Islands., I., *Philippine Jour. Sc.*, B, 1914, ix, 381–439.

Woldert, A., Cultivation of the Estivo-Autumnal Malarial Parasite in the Mosquito—*Anopheles quadrimaculata*, *Jour. Am. Med. Assn.*, 1901, xxxvi, 559–563.

Woldert, A., Original Specimens of Zygotes of Estivo-Autumnal Malarial Parasites in the Middle Intestine of the Mosquito (*Anopheles quadrimaculatus*), *Proc. Path. Soc., Philadelphia*, 1901, N.S., iv, 129–134.

## EXPLANATION OF PLATES.[1]

### PLATE 98.

FIG. 1. Oocysts of *Plasmodium falciparum* in *Anopheles crucians* (No. 703.6).
FIG. 2. Oocysts of *Plasmodium vivax* in *Anopheles punctipennis* (No. 612.1).

### PLATE 99.

FIG. 3. Two oocysts of *Plasmodium vivax* in *Anopheles punctipennis* (No. 612.1).
FIG. 4. Five oocysts of *Plasmodium vivax* in *Anopheles punctipennis* (No. 612.1).

### PLATE 100.

FIG. 5. Eight oocysts of *Plasmodium falciparum* in *Anopheles crucians* (No. 703.6).
FIG. 6. Ten oocysts of *Plasmodium falciparum* in *Anopheles crucians* (No. 703.6).

### PLATE 101.

FIG. 7. An oocyst of *Plasmodium falciparum* in *Anopheles punctipennis* (No. 617.4), showing formation of sporoblasts.
FIG. 8. Same as Fig. 7, more highly magnified.

### PLATE 102.

FIG. 9. An oocyst of *Plasmodium vivax* in *Anopheles punctipennis* (No. 614.1). This body contained active sporozoites and was at the point of rupturing.
FIG. 10. The empty capsule of an oocyst of *Plasmodium vivax* in *Anopheles punctipennis* (No. 612.5), after the escape of the sporozoites.
FIG. 11. Two empty capsules of oocysts of *Plasmodium vivax* in *Anopheles punctipennis* (No. 614.1).

### PLATE 103.

FIG. 12. A normal salivary gland of *Anopheles crucians*, showing the relation of the three lobes.
FIG. 13. A mass of sporozoites of *Plasmodium vivax* in *Anopheles quadrimaculatus* (No. 365.5). These were expelled from the salivary glands by the pressure of the cover glass.

---

[1] The author is greatly indebted to Dr. F. M. Johns for aid in the preparation of the microphotographs of infected mosquitoes, and to Dr. W. S. Thayer of Baltimore for confirmation of his interpretation of several of the preparations.

PLATE 104.

FIG. 14. Sporozoites of *Plasmodium falciparum* in glands of *Anopheles punctipennis* (No. 617.4). (Although a very poor illustration it has been included since it is the only microphotograph obtained of sporozoites in this species of mosquito.)

FIG. 15. Sporozoites of *Plasmodium vivax* in *Anopheles quadrimaculatus* (No. 373.5).

PLATE 105.

FIG. 16. Sporozoites of *Plasmodium vivax* in *Anopheles quadrimaculatus* (No. 365.1). This illustrates the typical clumping in one of the gland cells.

FIG. 17. Same as Fig. 16, more highly magnified.

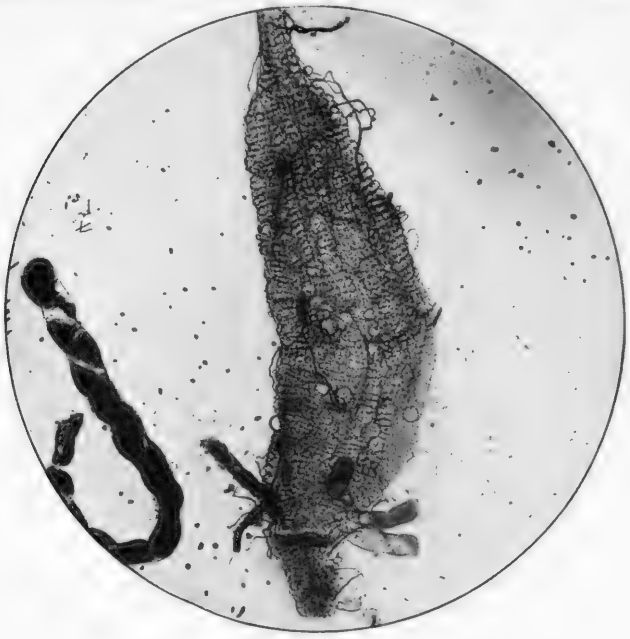


FIG. 1.

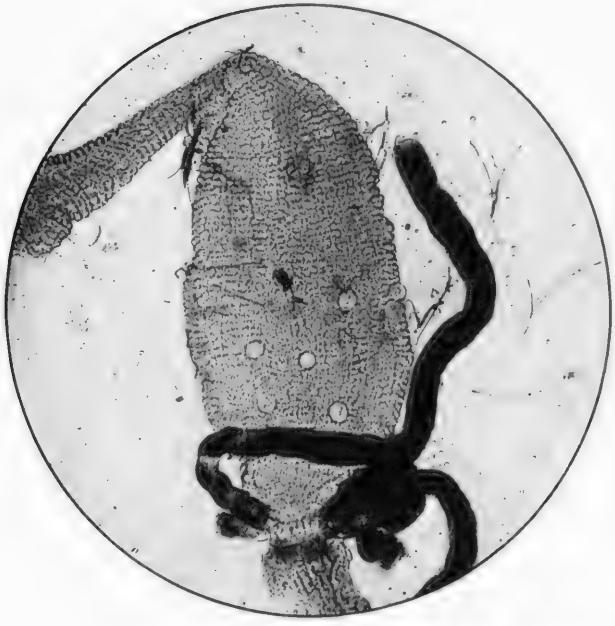


FIG. 2.

(King: Development of Malaria Parasites.)

FIG. 3.

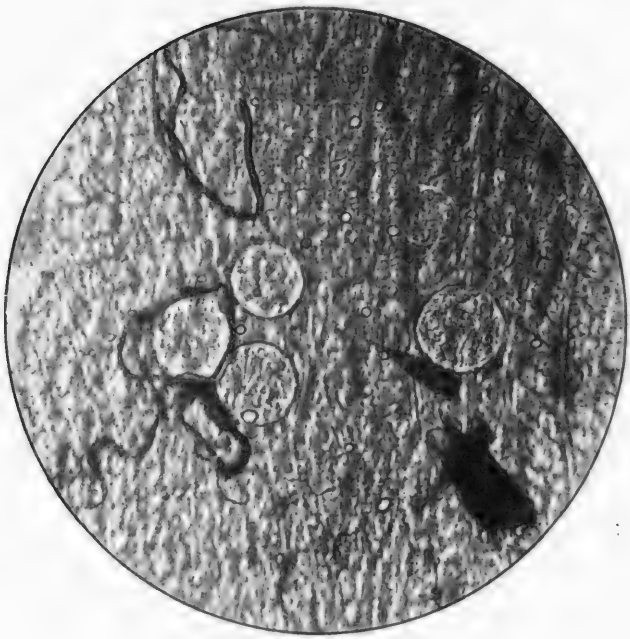


FIG. 4.

(King: Development of Malaria Parasites.)

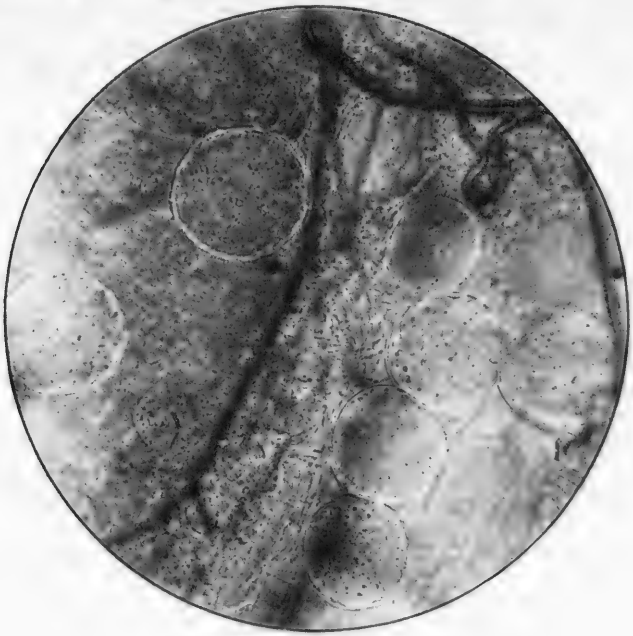


FIG. 5.

FIG. 6.

(King: Development of Malaria Parasites.)

FIG. 7.

FIG. 8.

(King: Development of Malaria Parasites.)

FIG. 9.

FIG. 10.

FIG. 11.

(King: Development of Malaria Parasites.)

FIG. 12.

FIG. 13.

(King: Development of Malaria Parasites.)

FIG. 14.

FIG. 15.

(King: Development of Malaria Parasites.)

FIG. 16.

FIG. 17.

(King: Development of Malaria Parasites.)

# INTESTINAL OBSTRUCTION.

## VI. A Study of Non-Coagulable Nitrogen of the Blood.

By J. V. COOKE, M.D., F. H. RODENBAUGH, M.D., and G. H. WHIPPLE, M.D.

(*From the George Williams Hooper Foundation for Medical Research, and the Department of Pathology of the University of California Medical School, San Francisco.*)

(Received for publication, March 3, 1916.)

This communication deals with analyses of the blood in intestinal obstruction, intestinal closed loops, and other acute intoxications. The tables give figures for non-coagulable nitrogen, urea nitrogen, and in some instances the total nitrogen partition in the blood. Our interest in this study of the blood was aroused by a communication of Tileston and Comfort (1), who in a large series of human cases reported three cases of intestinal obstruction with very high non-coagulable nitrogen. Since that time we have studied the blood of various experimental animals which were being observed in connection with other experimental work.

We found that most cases of intestinal obstruction, especially with signs of acute intoxication, showed a high non-coagulable blood nitrogen, and it seemed possible that this factor might be of value in diagnosis and especially prognosis of acute abdominal conditions. We are now convinced that this non-coagulable nitrogen determination is of value in various acute intoxications. If the reading is high, one may assume a dangerous grade of intoxication, but, on the contrary, one may not assume that a low reading gives evidence of slight intoxication, because a fatal outcome may be associated with a low reading. It is of considerable value to know that the non-coagulable nitrogen of the blood may show high readings in other conditions besides renal disease.

On the other hand, determinations of the blood urea alone are of somewhat less value in studying the retention products in the blood in these conditions. In our experimental animals the blood urea

has varied from less than 30 per cent to more than 80 per cent of the total non-coagulable nitrogen, and, while a high urea reading is the rule, the variations in the urea curve and the curves of the other non-coagulable nitrogenous substances are so great that the urea reading is a somewhat unreliable index of the extent to which non-coagulable nitrogenous substances have accumulated.

Our results are given below in the form of tables and some clinical and experimental data are given in addition, but it is not needful to give the entire experimental data at this time. Usually one experiment in each table is given in some detail as an example of the group.

### *Methods.*

Dogs were used in most experiments. A few cats were used, and one human case was included. All operations on animals were done under surgical anesthesia with the usual surgical technique.

The blood was taken in a glass syringe from the jugular vein and used for the various tests. When the blood was obtained at the time the animal was killed, it flowed from a cannula in the carotid. A few samples taken *in extremis* were obtained after anesthesia by opening the thorax and aspirating direct from the heart.

Non-coagulable nitrogen is determined as follows: With a volumetric pipette 10 cc. of blood are added to 65 cc. of distilled water, and 5 cc. of a 1 per cent sodium oxalate solution. The mixture is brought to a boil, gently rotating the flask while heating, and is then faintly acidified with acetic acid. A few crystals of sodium sulphate are added, the mixture is shaken, and, after the addition of 20 cc. of a 1 per cent solution of uranium acetate, is thoroughly shaken again and filtered. Nitrogen estimations by the Kjeldahl method are done in duplicate on 30 cc. samples of the clear filtrate,—each representing 3 cc. of blood,—and the non-coagulable nitrogen calculated for 100 cc. Creatinine and creatine are determined by the method of Folin (2) with a standard of creatinine zinc chloride. Urea is determined by the method of Marshall as modified by Van Slyke and Cullen (3). Uric acid is determined by the method of Benedict (4).

## EXPERIMENTAL OBSERVATIONS.

### *Simple Obstruction, Recovery, and Second Operation.*

*Dog 15–12.*—Mongrel, female; weight 40 pounds.

Mar. 8. A simple obstruction was made in the middle of the small intestine, and a small piece of tape was fixed about the small intestine to occlude its lumen but not to injure the intestinal wall.

Mar. 9. Dog quiet. Temperature 38.5°C. Slight vomiting.

Mar. 10. Dog shows usual picture of obstruction with vomiting. Temperature 38°C. Weight 38 pounds.

Mar. 11. Condition the same. Intoxication not striking. Weight 36 pounds.

Mar. 12. Condition the same. Weight 34 pounds. Late in the day the dog showed intoxication and small pulse. Infusion of 1,000 cc. of Locke's solution given in the jugular. Blood taken before and after infusion. The infusion caused clinical improvement.

Mar. 13. Dog still vomiting; the intoxication is not severe. Temperature 38.5°C. Weight 35.5 pounds. The marked drop in non-coagulable nitrogen is a striking feature of this experiment, and is difficult to explain, although the diuresis and replacement of lost fluid may be important factors. 1 p.m. Ether anesthesia and laparotomy. The obstruction was removed easily, and the intestinal wall closed over by sutures. At the end of the operation intravenous infusion of 1,000 cc. of Locke's solution. Dog made good recovery from operation.

Mar. 14. Dog vomited a little and refused food.

Mar. 15. Vomiting continues, and animal eats a little. Temperature 38.7°C. Weight 34 pounds. Given 1,000 cc. of Locke's solution intravenously.

Mar. 16. Much improvement; dog takes food; no vomiting. Weight 35 pounds.

Mar. 17 and 18. Continued improvement.

Mar. 19. Improvement continues; dog has passed feces.

Apr. 8. Dog appears normal. Second operation to establish a drained loop of duodenum including about 8 inches of the first part of the jejunum; distal portion of loop pulled through a puncture wound in left rectus; duodenum closed below pancreatic duct and gastrojejunostomy done.

Apr. 9. Dog sick, vomiting. Pulse weak. 3 p.m. Severe intoxication; much chocolate colored vomitus. Ether anesthesia. Killed.

*Autopsy.*—Thorax, heart, and lungs normal; abdominal viscera are normal; peritoneum is clean; gastro-enterostomy is patent; stomach shows slight engorgement of mucosa. Duodenum between pylorus and point of section shows a swollen and congested mucosa. It contains a blood-stained fluid. Jejunum below gastro-enterostomy shows engorgement of its mucosa very like that noted after lethal injection of a toxic proteose. The ileum and colon show congestion of their mucosæ to a less extent. The loop contains chocolate-red, slimy material; the mucosa shows no ulceration, but is congested and deep red; there are some submucous ecchymoses.

This experiment is of interest because of the recovery from the first obstruction. An obstruction was produced in the middle of the small intestine by means of a tape. On the 6th day the tape was removed at a second operation, and the dog returned to a normal

TABLE I.

*Dog 15–12. Simple Obstruction, Recovery, and Second Operation.*

| Day after operation. | Time. | Non-coagulable nitrogen.* | Remarks. Mongrel, female; weight 40 lbs. |
|---|---|---|---|
| | | *mg.* | |
| 2 | 2.00 | 23 | Vomited once. Pulse good. Temp. 38.5°C. |
| 3 | 11.00 | 24 | Dog vomiting. Condition good. 38 lbs. |
| 4 | 4.00 | 68 | Dog vomiting frequently. 36 lbs. Temp. 38°C. |
| 5 | 11.00 | 119 | Dog vomiting. Pulse good. Feces in cage. 34 lbs. Temp. 38°C. |
| 5 | 2.50 | 131 | Dog prostrated. Pulse tension low. Infusion of 1,000 cc. of Locke's solution. |
| 5 | 3.20 | 117 | Blood after infusion. Clinical improvement. |
| 6 | 11.30 | 31 | Dog improved. Vomiting less. |
| 2nd operation. | 1.00 | 42 | Laparotomy and removal of obstruction. Infusion of 1,000 cc. of Locke's solution. Blood at end of infusion. |
| 7 | 11.00 | 36 | Vomits occasionally. Refuses food. |
| 9 | 10.00 | 26 | Dog improving. 35 lbs. |
| 11 | 4.00 | 28 | Dog improving rapidly. 35.5 lbs. Temp. 38.4°C. |
| 12 | 11.00 | 31 | Dog passed feces. Seems quite well. |
| 32 | — | — | Normal. |
| 3rd operation. | 3.00 | — | Drained loop of jejunum. Long operation. |
| 33 | 12.00 | 56 | Dog much intoxicated and vomiting. Urea nitrogen 31 mg. |
| 33 | 3.00 | 71 | Animal acutely sick. Killed. Amino nitrogen 9.3 mg. |

* Non-coagulable nitrogen is given in terms of mg. per 100 cc. of blood.

condition. The third operation was performed, and about 18 inches of the lower duodenum and first portion of the jejunum were isolated as a drained loop opening in the left side. A gastro-enterostomy was performed. The animal showed grave intoxication with much vomiting and death in 24 hours. There was no peritonitis but some interference with the blood supply of the drained loop.

Table I shows the fluctuation in the blood non-coagulable nitrogen. There was a steady rise to a maximum of 131 mg. per 100 cc. of blood on the 5th day. The dog was given an infusion, and the following day the non-coagulable nitrogen had dropped to 31 mg. One is tempted to assume as a simple explanation that the replace-

ment of fluid and the consecutive diuresis sweep out these substances from the blood. This undoubtedly does occur, but the story is not quite as simple as this. For example, in other experiments with a grave intoxication a transfusion may not affect in the least the level of blood non-coagulable nitrogen, in spite of a striking diuresis. There is no evidence to point to lack of eliminative power of the kidneys as a factor.

TABLE II.

*Simple Obstruction.*

| Dog No. | Day after operation. | Non-coagulable nitrogen.* | Urea nitrogen.* | Weight. | Temp. | Remarks. |
|---|---|---|---|---|---|---|
| | | *mg.* | *mg.* | *lbs.* | *°C.* | |
| 15–20 | 6 | 48 | — | 15.3 | 38.5 | No vomitus. Dog quiet. |
| | 7 | 33 | — | 14.5 | 38.4 | Condition the same. |
| | 8 | 38 | — | 14.3 | 39.1 | " " " |
| | 9 | 40 | 19 | 13.8 | 38.5 | Little vomitus, intoxication moderate. Killed. |
| 15–26 | 1 | 77 | — | — | — | Blood after operation. |
| | 3 | 46 | 34 | 42.8 | 39.2 | Vomiting and sick. |
| | 4 | 60 | 35 | 41.3 | 39.2 | Condition the same. |
| | 5 | 56 | 40 | 40.8 | 39.3 | " " " |
| | 6 | 60 | 39 | 40.0 | 39.1 | " " " |
| | 7 | 94 | 58 | 39.0 | 39.6 | Dog weak. |
| | 8 | 79 | 42 | — | — | Dog seems better. Killed. Slight chronic nephritis. |
| 16–45 | 1 | 34 | 15 | 36.0 | — | Blood before operation. |
| | 3 | 52 | 28 | 34.0 | 39.7 | Vomiting large amounts. |
| | 4 | 59 | 21 | 33.0 | 39.3 | Vomiting. |
| | 6 | 90 | 43 | — | — | Dog acutely sick. Killed. |
| 16–56 | 1 | — | 22 | 22.0 | — | |
| | 4 | — | 22 | 19.0 | 38.0 | Dog vomiting since operation. |
| | 5 | 63 | 21 | — | 37.7 | 11 a.m. Animal in fair condition. Proteose injection. Blood before injection. |
| | 5 | 81 | 27 | 19.5 | — | 11.10 a.m. Recovered from proteose injection in 6 hrs. Died in night. Blood after injection. |

* Non-coagulable and urea nitrogen are given in terms of mg. per 100 cc. of blood.

*Simple Obstruction for 5 Days. Toxic Death.*

*Dog 16–45.*—Shepherd, mongrel, female; weight 36 pounds.

Nov. 10. Simple obstruction in the middle of the small intestine by means of complete section and an inversion of cut ends.

Nov. 11 and 12. Dog is vomiting, and there is a steady loss in weight.

Nov. 13. Much thin yellow vomitus. Temperature 39.3°C. Weight 33 pounds.

Nov. 15. 9 a.m. Dog is severely intoxicated. Ether anesthesia. Killed. The readings for non-coagulable nitrogen and urea nitrogen are given in Table II.

*Autopsy.*—Thorax, lungs, and heart normal. The viscera show nothing of interest except a slight congestion. The peritoneum is dry and clean. There are a few fibrinous adhesions about the site of obstruction. Intestinal tract below obstruction is collapsed and mucosa normal. The intestine above the obstruction shows only slight dilatation. There is no congestion of the mucosa and no ulceration. The stomach shows come congestion of the cardia. The material above the obstruction consists of thin yellow material, like pea-soup. 160 cc. in amount. Kept for further study.

The protocol of Dog 16–45 gives a typical story of an uncomplicated, untreated case of simple obstruction of the middle of the small intestine. Vomiting is constant, but the grave intoxication does not appear until the 5th day, when the non-coagulable nitrogen is found to be high—90 mg. per 100 cc. of blood. The autopsy findings are characteristic, and the peritoneum is clean.

Table II shows a considerable variety of non-coagulable nitrogen readings. The last three experiments show a definite rise in non-coagulable nitrogen above normal, and this is the rule. The first experiment, however, is a good example of a dog which maintained practically a normal level in spite of a prolonged obstruction. It should be kept in mind that this dog had a resistance considerably above normal, consequently was not as acutely intoxicated, and was killed 8 days after operation while still in fair condition. A similar state of affairs is noted in closed loops of the intestine, and with a slowly progressing intoxication the non-coagulable nitrogen may scarcely rise above its normal level.

This table of simple obstruction experiments gives also the urea nitrogen of the blood. It is noted at once that these intoxications give different urea readings with high non-coaguable nitrogen than is found in chronic nephritis. Urea nitrogen in nephritis usually

constitutes more than 50 to 60 per cent of the total nitrogen, but here it is seen that it often falls below 50 per cent. The residual or undetermined nitrogen in these experiments is constantly high. We suspect that this is a fairly constant feature in various proteose intoxications.

TABLE III.

*Closed Loop of Duodenum and Jejunum. Gastro-Enterostomy.*

| Dog. No. | Day after operation. | Non-coagulable nitrogen.* | Remarks. |
|---|---|---|---|
| | | *mg.* | |
| 15–2 | 2 | 41 | Condition good, vomiting. |
| | 3 | 89 | " " |
| | 4 | 113 | Grave intoxication; subnormal temperature. Killed. |
| 15–9 | 1 | 51 | Blood taken at time of operation. Weight 33 lbs. |
| | 2 | 103 | Dog sick, listless, vomiting. Died during night. |
| 15–10 | 1 | 20 | Blood at operation. 23 lbs. |
| | 2 | 35 | Dog toxic. Pulse good. |
| | 3 | 70 | Dog in fair condition. Intoxication moderate. |
| | 4 | 135 | Dog toxic. Pulse weak. Killed. |
| 15–14 | 1 | 40 | Blood before operation. 30 lbs. |
| | 2 | 33 | Dog vomiting. Slightly toxic. |
| | 3 | 37 | " " Moderately toxic. 28.5 lbs. |
| | 4 | 53 | Little vomiting. Condition the same. |
| | 5 | 55 | 11 a.m. Dog sick. Muscular tremors. 26.5 lbs. |
| | 5 | 65 | 2.30 p.m. Killed. Amino nitrogen 5.7 mg. per 100 cc. |

* Non-coagulable nitrogen is given in terms of mg. per 100 cc. of blood.

### *Long Loop of Duodenum and Jejunum. Gastro-Enterostomy.*

*Dog 15–10.*—Long haired mongrel, male; weight 23.5 pounds.

Mar. 3. Isolated a long loop including lower half of duodenum and upper part of the jejunum; occlusion was effected by means of tapes applied as usual; gastro-enterostomy performed.

Mar. 4. Dog intoxicated. Temperature 38.5°C.

Mar. 5. Condition unchanged.

Mar. 6. Dog very sick; much vomiting. Pulse pressure low. Slow respiration. Temperature 37.3°C. Ether anesthesia and bleeding from carotid, which showed an arterial pressure scarcely above the usual venous pressure.

*Autopsy.*—Thorax normal. Peritoneum is clean and dry, except for a few bits of fibrin close to the site of operation. Liver shows a little congestion.

Other organs are normal. The loop is completely isolated from the intestine; it contains about 50 cc. of creamy, white, syrupy material having the characteristic odor. The mucosa shows a very slight amount of congestion, no ulceration, and is intact throughout. Under the microscope it appears normal. Loop fluid preserved for further study.

The protocol of Dog 15–10 (Table III) is a good example of uncomplicated closed intestinal loops of a certain type. It will be seen that the non-coagulable nitrogen of the blood rises regularly with the intoxication developing under these conditions. It must not be forgotten that these loops include the lower half of the duodenum and much of the upper jejunum. The ends of the loop are closed by tapes, and the contents of the upper duodenum must be forced back into the stomach where they gain access to the jejunum by a posterior gastrojejunostomy. This experiment, therefore, causes an obstruction to the first half of the duodenum, and this is of no small importance, as is seen on comparing these experiments with other closed loops of a different sort in Table V. We intend to take up this point in another communication.

TABLE IV.

*Closed Loop of Jejunum. Gastro-Enterostomy.*

| Dog No. | Day after operation. | Non-coagulable nitrogen.* | Remarks. |
|---|---|---|---|
| | | *mg.* | |
| 15–29 | 2 | 23 | Blood at end of infusion. Intoxication definite. Infusion of 1,000 cc. of 1% dextrose solution. Weight 35 lbs. |
| | 4 | 35 | Vomiting. Pulse fair. |
| | 5 | 39 | Dog weak. Blood taken before infusion. 1,000 cc. of 10% dextrose. 32 lbs. |
| | 6 | 98 | 5 p.m. Infusion of 1,000 cc. of 10% dextrose. 11 p.m. Animal moribund. Killed. Loop rupture. |
| 15–45 | 4 | 30 | Dog slightly intoxicated. 22 lbs. |
| | 5 | 76 | 10 a.m. Dog about the same. |
| | 5 | 115 | 5 p.m. Dog looks sick. Pulse weak. Killed. Early peritonitis. |
| 15–19 | 1 | 31 | Blood at end of operation. 22 lbs. |
| | 2 | 62 | Dog very quiet. Died during night. Acute intoxication. |

* Non-coagulable nitrogen is given in terms of mg. per 100 cc. of blood.

*Long Loop of Jejunum with Rupture and Peritonitis.*

*Dog 15–29.*—Adult spaniel, male; weight 35 pounds.

Apr. 23. Isolated a long loop including the lower portion of the duodenum and about 3 feet of the jejunum, using tapes. Gastrojejunostomy performed as usual.

Apr. 24. Dog is sick. There is much dark brown vomitus. Pulse poor, and for this reason infusion of normal salt solution plus 1 per cent dextrose was given intravenously, followed by much improvement.

Apr. 25. Intoxication not striking.

Apr. 26. Much vomiting. Weight 33 pounds. Temperature 38.5°C.

Apr. 27. Dog seems sick. Weight 32 pounds. Temperature 38.4°C. 12 m. Infusion of 1,000 cc. of 10 per cent dextrose. 5 p.m. Dog not much improved. Given a second infusion of 1,000 cc. of 10 per cent dextrose.

Apr. 28. Much vomiting. Pulse tension low. Temperature 38.4°C. Weight 31.3 pounds. 5 p.m. Infusion of 1,000 cc. of 10 per cent dextrose. Dog is vomiting constantly. 11 p.m. Dog very sick. Ether anesthesia. Killed.

*Autopsy.*—The peritoneal cavity contains 500 cc. of blood-tinged loop fluid of the usual appearance. The peritoneum is specked with ecchymoses, but there is no fibrin. Rupture took place at the site of the lower ligature, which cut through the wall of the intestine. Upper ligature is tight. Death took place promptly from absorption of the toxic material poured from the loop into the peritoneum. The organs show some congestion associated with intoxication by means of loop fluid. Loop is empty. It shows congestion and some areas of submucous hemorrhage due undoubtedly to acute distention.

The protocol of Dog 15–29 (Table IV) shows the same rise in non-coagulable blood nitrogen noted in the closed intestinal loop experiments of Table III. These experiments show various complications met with in these loop experiments,—peritonitis, rupture of loop, and overwhelming intoxication. When the intoxication is very acute and severe, the infusion of normal salt or dextrose solutions will not depress the level of non-coagulable nitrogen in the blood.

*Long Loop of Jejunum. Rupture in 20 Days.*

*Dog 16–22.*—Fox-terrier, male; weight 18.5 pounds.

Sept. 28. Isolated a long loop of jejunum; ends of loop sectioned and turned in; the jejunum joined around the loop by end to end anastomosis to establish direct continuity of intestinal lumen.

Sept. 29. Dog looks well; no vomiting. (See Table V for details of temperature, weight, and non-coagulable and urea nitrogen). There was steady loss of weight during the next week with occasional attacks of vomiting; at times the dog eats a little food.

TABLE V.

*Long Loop, Jejunum, Ileum.*

| Dog No. | Day after operation. | Non-coagulable nitrogen.* | Urea nitrogen.* | Weight. | Temp. | Remarks. |
|---|---|---|---|---|---|---|
| | | *mg.* | *mg.* | *lbs.* | *°C.* | |
| 16–20 | 1 | 49 | 9 | 39.0 | — | Before operation. Long loop jejunum. |
| | 1 | 50 | 8 | — | — | After operation. |
| | 3 | 60 | 4 | — | 40.0 | Condition good. |
| | 4 | 28 | 7 | — | 40.0 | Dog has vomited. |
| | 5 | 35 | 11 | 37.5 | 40.1 | No vomiting. |
| | 8 | 24 | 8 | 36.8 | 40.1 | " " |
| | 9 | 22 | 6 | 37.3 | — | Condition improved. |
| | 10 | 28 | 8 | 37.5 | 39.8 | Dog in good condition. |
| | 11 | 28 | 8 | 36.5 | 39.7 | " " " " |
| | 13 | 33 | 12 | 35.3 | — | Dog vomiting and intoxicated. |
| | 14 | 53 | 16 | — | — | Dog vomiting. Infusion of 1,000 cc. of 7% dextrose solution. |
| | 15 | 51 | 18 | 33.0 | 39.3 | Dog vomiting, weaker. Death 18th day. |
| 16–22 | 1 | 22 | 10 | 18.5 | — | Before operation. Long loop jejunum. |
| | 3 | 36 | 8 | 18.0 | 39.4 | Intoxicated. No vomiting. |
| | 4 | — | 16 | 17.5 | — | Vomiting, large amount. |
| | 5 | 35 | 19 | 17.3 | 39.3 | Little vomiting. |
| | 7 | 30 | 14 | 15.8 | 38.9 | " " Loss of strength. |
| | 9 | 36 | 10 | 15.5 | 39.1 | No vomiting. |
| | 11 | 35 | 12 | 15.5 | 39.1 | Solid feces. |
| | 14 | 36 | 6 | 15.3 | — | Condition the same. |
| | 15 | 48 | 9 | 15.0 | — | No vomiting. |
| | 16 | 43 | 10 | 14.5 | — | Animal looks intoxicated. |
| | 20 | 41 | 20 | 13.7 | 38.8 | Dog weak. Infusion of 1,000 cc. of 7% dextrose. |
| | 21 | 64 | 29 | — | — | Dog sick. Killed. |
| 16–37 | 5 | 90 | 21 | 27.3 | 38.9 | Vomiting for past 3 days. Long loop of ileum. |
| | 6 | 45 | 20 | 27.3 | 39.1 | Dog improved; no vomiting; eating. |
| | 10 | 53 | 20 | 25.8 | 38.9 | Some diarrhea. |
| | 11 | — | 13 | — | — | Dog looks well. |
| | 13 | 59 | 21 | 24.3 | — | Condition the same. |
| | 14 | 57 | 25 | 24.0 | — | Dog improved. |
| | 15 | 31 | 19 | 23.8 | — | Passed solid stool. Eating. |
| | 17 | 54 | 32 | 24.0 | 38.9 | Peristalsis visible in abdomen. |
| | 18 | 40 | 19 | 24.0 | 39.3 | Dog losing ground. |
| | 19 | 40 | 9 | 23.8 | 38.9 | Condition good. Killed. |

* Non-coagulable and urea nitrogen are given in terms of mg. per 100 cc. of blood.

Oct. 14. Dog has passed formed stools. No vomitus. Weight 14.5 pounds. There is evidence of chronic intoxication. Dog was injected with a toxic proteose obtained from ox pancreas; dog was slightly intoxicated by this proteose, while a normal control animal was fatally poisoned in 11 hours by a similar dose.

Oct. 15. Dog looks well; no vomitus.

Oct. 18. Dog is very sick. Ether anesthesia. Killed.

*Autopsy.*—Thorax, heart, and lungs normal; spleen, kidney, pancreas, etc., normal; liver rather atrophic; no signs of fatty degeneration. Peritoneal cavity contains a good deal of the loop fluid which has escaped from a recent rupture. There is little reaction in the peritoneum as the intoxication was so acute. Stomach and intestine outside of loop show congestion of the mucosa so common in proteose intoxication. Loop is made up of two parts isolated by old adhesions. One-half is slightly collapsed due to escape of fluid into the peritoneum; there is an ulcer in the wall, which has perforated the mucosa; all this portion is somewhat red and swollen, and shows two other ulcers involving the mucosa. The other half of the loop consists of three small coils twisted about its mesenteric attachment; adhesions have caused some constriction of mesenteric vessels giving engorgement of the loop but not infarction; this portion of the loop contains a pale, slate colored fluid with no blood; the mucosa is swollen and engorged but shows no hemorrhage; the walls of the loop are everywhere hypertrophied.

The protocol of Dog 16–22 (Table V) shows a different type of closed intestinal loop with a slowly progressing intoxication and relatively slight changes in the non-coagulable nitrogen of the blood. The loops of the jejunum or ileum are isolated completely by cross section of the gut in two places. The loop is made by turning in the ends of the isolated portion of the intestine or by doing an end to end anastomosis thus forming a circle out of the isolated gut. The continuity of the rest of the intestine is established by means of an end to end anastomosis which gives an unobstructed flow from duodenum to jejunum and ileum. This does away with the obstruction in the first half of the duodenum which is present in the loops isolated by ligature and gastrojejunostomy (Tables III and IV). The difference in severity of intoxication under these conditions is obvious.

The urea nitrogen readings in general show a low percentage value of the total non-coagulable nitrogen. The weight curve shows the gradual loss in body weight due to the chronic intoxication, even in the absence of vomiting.

The immunity to proteose injection (Dog 16–22) shown by a dog with a long standing closed intestinal loop indicates that the presence

of a closed loop of intestine causes a chronic proteose intoxication which gives a certain degree of immunity against poisoning by various foreign proteoses. This point will be taken up in another communication and the experiments will be given in detail.

TABLE VI.

*Long Loop, Jejunum, Ileum, Complications.*

| Dog No. | Day after operation. | Non-coagulable nitrogen.* | Urea nitrogen.* | Weight. | Temp. | Remarks. |
|---|---|---|---|---|---|---|
| | | *mg.* | *mg.* | *lbs.* | *°C.* | |
| 16–42 | 3 | 32 | 13 | 21.0 | 39.1 | Long loop jejunum, obstruction, peritonitis. |
| | 4 | 39 | 15 | 20.5 | 39.0 | Considerable vomiting. |
| | 5 | 39 | 13 | 20.7 | 38.8 | " " Animal looks sick. |
| | 6 | 50 | 22 | 21.0 | 37.9 | 9.30 a.m. No vomitus. |
| | 6 | 56 | 39 | — | 34.5 | 4 p.m. Dog toxic. Killed. |
| 16–48 | 4 | 30 | 14 | 19.5 | 39.0 | Long loop jejunum, terminal obstruction, and volvulus. |
| | 6 | 45 | 19 | 19.0 | 39.2 | Condition good. No vomiting. |
| | 8 | 32 | 15 | 19.3 | 38.8 | Condition the same. |
| | 12 | 46 | 22 | 19.5 | 39.3 | " " " Found dead on 13th day. |
| 16–57 | 1 | 58 | 23 | 18.5 | — | Short loop of ileum. General peritonitis. |
| | 6 | 44 | 11 | 15.5 | 39.3 | Dog has been in good condition. |
| | 8 | 166 | 51 | 15.5 | 36.8 | Some vomiting. Animal moribund. Killed. |
| 16–71 | 1 | 36 | 12 | 27.5 | — | Long loop of jejunum. General peritonitis. |
| | 2 | 36 | 12 | 26.3 | 38.3 | Dog in fair condition. |
| | 4 | 53 | 31 | — | — | Animal moribund. Killed. |
| 16–39 | 1 | — | 15 | 24.5 | — | Long loop of jejunum. General peritonitis. |
| | 3 | 71 | 18 | 23.5 | 38.8 | Much vomiting. Pulse poor. Infusion of 1,000 cc. of 5% dextrose. Blood at end of infusion. Death next day. |

* Non-coagulable and urea nitrogen are given in terms of mg. per 100 cc. of blood.

*Long Loop of Jejunum. Obstruction. General Peritonitis.*

*Dog 16–42.*—Mongrel, male; weight 22 pounds.

Nov. 6. Isolated a long loop of jejunum by section and inversion of ends; jejunum joined around the loop by means of end to end anastomosis between upper and lower end.

Nov. 8. Dog pretty well. Temperature 39.2°C. Weight 22 pounds.

Nov. 9. Much vomiting.

Nov. 10. Dog seems better. (See Table VI for details.)

Nov. 11. Little vomiting. Temperature 37.9°C. Weight 21 pounds. 4 p.m. Dog is very weak. Temperature 34.5°C. Ether anesthesia. Killed.

*Autopsy.*—Thorax, heart, and lungs normal. Peritoneal cavity contains many isolated pockets of purulent exudate. A large round worm is free in the peritoneal cavity, evidently having escaped from the loop, which contains numerous similar worms. There are organized adhesions just below the end to end anastomosis causing a sharp kink and probably complete obstruction. Duodenum and jejunum contain the usual obstruction fluid. The loop contains 130 cc. of pale, slate colored fluid with a strong odor. There are numerous live and active round worms in the loop. No point can be found where the worm escaped from the loop. The peritonitis is probably of 1 or 2 days' duration.

The protocol of Dog 16–42 (Table VI) shows some of the complications which may arise in the closed loop experiments. Peritonitis is most common, but volvulus and obstruction may occur. These complications do not modify the picture, and we have good evidence that peritonitis alone may be associated with a high non-coagulable nitrogen. It is of interest to note that we have been able to isolate a toxic proteose from peritoneal exudates, and we believe that this proteose is of considerable importance in explaining the intoxication of general peritonitis. The rise in non-coagulable nitrogen in the blood may be in part due to the proteose intoxication.

*Proteose Injection. Rise in Non-Coagulable Nitrogen.*

*Dog 15–50.*—Small black and tan, male; weight 13 pounds.

12 m. (A) Blood non-coagulable nitrogen 33 mg. per 100 cc. of blood; ether anesthesia and intravenous injection of purified proteose obtained from closed intestinal loops. Kymograph observation during slow injection of 65 cc. of solution of proteose which had been reprecipitated first by alcohol and then by half saturation with ammonium sulphate as described previously (5). There was a little fall in blood pressure.

12.45 p.m. After injection (B) blood non-coagulable nitrogen 40 mg. per 100 cc. of blood.

3.15 p.m. (C) Blood non-coagulable nitrogen 80 mg. per 100 cc. of blood.

4 p.m. Death with prostration and subnormal temperature.

*Autopsy.*—There is the typical splanchnic engorgement due to fatal proteose intoxication. Spleen and liver are swollen and purple. Mucosa of duodenum and jejunum is swollen and deep reddish purple.

*Dog 15–51.*—Small fox-terrier, female; weight 15.5 pounds.

12 m. (A) Blood non-coagulable nitrogen 39 mg. per 100 cc. of blood. Ether anesthesia and intravenous injection of purified proteose obtained from closed intestinal loops. Kymograph observation during injection of 50 cc. of fluid showed no change in blood pressure.

12.30 p.m. After injection (B) blood non-coagulable nitrogen 32 mg. per 100 cc. of blood.

5 p.m. (C) Blood non-coagulable nitrogen 58 mg. per 100 cc. of blood.

Dog is prostrated; much vomiting and diarrhea.

Died in night.

*Autopsy.*—The findings are characteristic of proteose intoxication. There is much blood-tinged fluid in the intestines, and the mucosa is red and swollen.

These two experiments (Dogs 15–50 and 15–51) are of considerable interest, and show that acute poisoning with a pure proteose may cause the blood non-coagulable nitrogen to double in amount in 3 hours; for example, a rise from 40 to 80 mg. The proteose used in these experiments was pure, and not over 200 mg. were injected. The addition of this to the blood itself could not be detected by any method in use, and the method used by us causes precipitation of all primary proteoses at least. The great rise in non-coagulable nitrogen obviously must be explained by disintegration of body or tissue protein. This is of importance in explaining the high non-coagulable nitrogen associated with the closed loops of intestine or intestinal obstruction. In both instances we are dealing with a proteose intoxication, and we believe that much of the increase in blood non-coagulable nitrogen is due to disintegration of the tissues of the body. Catabolism, in other words, must be responsible for much of the non-coagulable nitrogen rather than retention.

Tables VII and VIII give the nitrogen partition of the total non-protein nitrogen of the blood expressed in mg. per 100 cc. of blood. One control nephritis gives a residual nitrogen of 11 per cent and 82 per cent urea nitrogen. A second case (Dog 16–49 in Table VIII) shows a similar picture. This experiment presents isolation of the bladder and implantation of the ureters into the intestine. There

TABLE VII.

*Intestinal Loops. Peritonitis and Obstruction.*

| No. | Non-coagulable nitrogen. | Urea nitrogen. | | Amino nitrogen. | Uric acid nitrogen. | Creatine nitrogen. | Creatinine nitrogen. | Residual nitrogen. | | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|
| | *mg.* | *mg.* | *per cent* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *per cent* | |
| Dog 16–37 | 40 | 9 | 23 | 1.4 | 0.6 | 0.6 | 1.5 | 26.9 | 67 | Long loop of ileum. Killed 19th day. |
| " 16–42 | 56 | 39 | 69 | 4.5 | 0.6 | 1.0 | 5.6 | 5.3 | 9 | Long loop of jejunum. Peritonitis, obstruction. |
| " 16–57 | 166 | 51 | 30 | 6.0 | 0.6 | 0.8 | 11.0 | 96.6 | 58 | Long loop of ileum. Peritonitis. |
| " 16–71 | 53 | 31 | 58 | 3.7 | 1.1 | 0.8 | 7.3 | 10.1 | 19 | Long loop of jejunum. Peritonitis. |
| " 16–80 | 40 | 28 | 70 | 3.9 | 1.0 | 0.3 | 3.1 | 3.7 | 9 | Long loop of ileum. Peritonitis. |
| " 15–45 | 115 | 77 | 60 | — | — | 0.8 | 5.9 | — | — | Long loop of jejunum. |
| Cat 16–7 | 296 | 137 | 46 | 6.8 | 2.3 | 0.7 | 21.3 | 127.9 | 43 | Long loop of jejunum and obstruction. Death in 4 days. |
| Dog 16–45 | 90 | 43 | 47 | 5.5 | — | — | — | — | — | Simple obstruction. Death in 5 days. |
| " 16–82 | 47 | 17 | 36 | 2.5 | 1.4 | 0.2 | 3.3 | 22.6 | 48 | Simple obstruction. Killed in 7 days. |
| Case 1 (Man) | 164 | 107 | 65 | 2.3 | 0.9 | 0.3 | 10.2 | 35.2 | 21 | Simple obstruction. Pneumonia. Death in 5 days. |

Nitrogen is given in terms of mg. per 100 cc. of blood.

was some obstruction to the outflow of urine and some escape into the peritoneum with an irritant peritonitis and absorption consequent to this. Here there are two factors—retention or lack of elimination plus peritonitis.

The tables show a high per cent of residual nitrogen or a low relative per cent of urea nitrogen. This is true particularly in the severe proteose intoxication with high non-coagulable nitrogen. Similar high readings in cases of nephritis and retention will scarcely show such high percentages of residual nitrogen. For this reason we be-

TABLE VIII.

*Bladder Isolation, Chronic Nephritis, Peritonitis, etc.*

| Dog No. | Non-coagulable nitrogen. | Urea nitrogen. | | Amino nitrogen. | Uric acid nitrogen. | Creatine nitrogen. | Creatinine nitrogen. | Residual nitrogen. | | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|
| | *mg.* | *mg.* | *per cent* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *per cent* | |
| 16–49 | 130 | 91 | 70 | 2.3 | 0.3 | 0.4 | 7.1 | 28.9 | 22 | Bladder isolation. Urine escape. Peritonitis. |
| 16–61 | 368 | 304 | 82 | 6.8 | 2.3 | 1.0 | 12.0 | 41.9 | 11 | Chronic nephritis. Old dog. Hemorrhagic gastritis. |
| 16–62 | 72 | 42 | 58 | 4.2 | 1.0 | 0.5 | 1.1 | 23.2 | 32 | Acute distemper. |
| 16–65 | 36 | 18 | 50 | 2.8 | 0.3 | 0.6 | 5.2 | 9.1 | 25 | Drained loop of ileum. Subcutaneous abscess. |
| 16–67 | 40 | 16 | 40 | 1.5 | 1.8 | 0.6 | 3.8 | 16.3 | 40 | Skin incisions. Mild distemper. |
| 16–69 | 164 | 58 | 38 | 4.2 | 1.2 | 0.9 | 5.3 | 94.4 | 57 | General peritonitis. |
| 16–85 | 67 | 29 | 43 | 3.1 | 0.4 | 0.7 | 3.6 | 30.2 | 45 | Distemper. Pneumonia. |

Nitrogen is given in terms of mg. per 100 cc. of blood.

lieve that the non-coagulable nitrogen determination gives more information than does urea nitrogen, and is of more clinical value in diagnosis and prognosis.

The blood content of amino nitrogen and uric acid nitrogen is low normal, and shows fluctuations within normal limits. Ammonia nitrogen was done in the majority of experiments but the method is inaccurate and the values are probably too high. It seemed best to leave these analyses out of the table and assume that ammonia nitrogen is included in the urea nitrogen. The difference scarcely exceeds 1 mg. at the most.

The creatine fraction is constantly low, but it is of interest to note that the creatinine nitrogen may be very high in many of these proteose intoxications, particularly in intestinal obstruction and closed intestinal loops. There are many readings of more than 5 mg. per 100 cc. of blood, and one experiment (Table VII, Cat 16–7) shows a reading of 21.3 mg. This occurred in a cat with intestinal obstruction plus a closed intestinal loop with a non-coagulable nitrogen of 296 mg. per 100 cc. of blood. The kidneys in all these cases,

unless otherwise noted, were normal. It is to be recalled that all these animals were sick and refused food—in fact the majority were vomiting more or less. A human case of intestinal obstruction and pneumonia shows a very high creatinine value, 10.2 mg., but autopsy showed practically normal kidneys.

*Human Intestinal Obstruction. Death in 5 Days.*

*Case I.*—C. K., German, male, 68 years of age.

*Past History.*—Negative.

*Present Illness.*—Began Jan. 16, 1916, with indefinite pain. No bowel movements since this time.

Jan. 17. A good deal of epigastric pain with vomiting, which became fecal in type the following day. Patient given castor oil and salts without results.

Jan. 18. Condition unchanged, except that vomiting became more severe.

Jan. 19. Entered hospital. At this time he had marked abdominal tenderness, and was vomiting frequently. Vomitus fecal in odor, watery, with fine brownish precipitate. Blood obtained showed very high non-coagulable nitrogen. Patient given 900 cc. of 6 per cent glucose with sodium carbonate intravenously. Infusion improved condition of patient.

*Blood Examination.*—White blood cells 18,400; hemoglobin 90 per cent.

3 p.m. Laparotomy and abdominal exploration. A portion of greatly congested intestine, about 10 cm., was found; the wall was edematous. It was placed back in the abdominal cavity. Abdomen closed. Volvulus(?).

Jan. 20. 10 a.m. Blood again obtained, and showed some decrease in non-coagulable nitrogen; vomiting not so marked; considerable fecal matter obtained by means of an enema. Pulse not good. 4 p.m. Blood again obtained; showed rising non-coagulable nitrogen. Given infusion of 750 cc. of 6 per cent glucose intravenously.

Jan. 21. 6 a.m. Patient died in stupor. Blood obtained by cardiac puncture a few minutes after death.

Autopsy performed 5 hours after death.

*Anatomical Diagnosis.*—Intestinal obstruction; volvulus (?); operation wound for relief of obstruction; early infarction and necrosis of loop of ileum; early serofibrinous peritonitis; bronchopneumonia (pseudolobar) of both lungs; acute hemorrhagic bronchitis; cloudy swelling of viscera.

The abdomen is considerably distended and tense. On incision a small amount of blood-tinged, slightly turbid fluid is found between the coils of an enormously distended small intestine. The loops are somewhat glued together by dry, plastic exudate. The surface of the intestines is somewhat dry and very definitely injected, more especially in the region of the abdominal incision. There are no adhesions except over the spleen. There are no hernial openings to be found in the pelvis or inguinal region. One of the stitches in the abdominal wound has caught

and firmly held a bit of omentum. One segment of the intestine, dark red in color and considerably swollen, measuring about 15 cm. in length, is found close to the liver. The swelling involves the wall of the intestine and the mesentery to a distance of about 5 cm. from the intestinal attachment. There is a clean-cut line of demarcation between the relatively normal but elongated mesentery and the short, inflamed, edematous mesenteric portion close to the intestine. The line of demarcation on the intestine is quite sharp, particularly at the upper end. It appears as though a band, or definite tight constriction, had been drawn about the portion of the ileum including the small part of the mesentery, shutting off a considerable part of the blood supply. This may have been due to a twist of the relaxed elongated mesentery. This was evidently relieved at operation, but the circulation did not establish itself properly owing to tissue injury, and the general appearance was that suggesting early hemorrhagic infarction. The picture, however, was not complete. Careful section of the mesenteric veins showed them to be quite free from thrombi even in their finer branches. There may be some very small thrombi in the smaller branches close to the mesenteric border, but these could not be dissected out.

*Lungs.*—The right lung weighs 870, and the left 560 gm. The lungs are voluminous, cushiony in their anterior portions; they are heavy and consolidated posteriorly. The pulmonic vessels are clear. The bronchi are intensely inflamed, their mucosa is velvety and purple, and they contain much serous blood-tinged fluid. On section the anterior portions are dry and cushiony. The posterior portions are consolidated and very moist. One can scrape off purulent material. The consolidation involves the posterior portion of the left upper and the greater portion of the right upper lobe and part of the right middle lobe. These areas of consolidation are mottled gray and purplish red. Some of the gray areas are very soft, and creamy material can be scraped off, indicating a beginning resolution of the exudate. This pneumonic process must have been of several days duration—estimated 2 to 3 days.

*Kidneys.*—Capsule comes off easily leaving a smooth surface, but for two retention cysts. There is one large retention cyst in the upper pole of the right kidney measuring about 2 cm. in diameter. Its wall contains a few thin plaques of calcified material. The kidney parenchyma on section appears normal. The pelvis is normal.

*Microscopic Examination. Kidneys.*—There are a few hyaline casts in some of the tubules, also a few hyaline scars in the cortex. In general the parenchyma looks normal except for definite cloudy swelling of the epithelium lining the convoluted tubules. There is no epithelial necrosis. The stroma of the pyramids shows a little edema.

*Lungs.*—The patches of pneumonia show the alveoli filled with an exudate of coagulated serum, mono- and polynuclear cells, fibrin, and enormous numbers of bacteria. In places the bacteria form almost a solid mat of rods showing capsules. The great overgrowth of bacteria is the striking feature of this lung.

*Mesentery.*—The swollen hemorrhagic area shows a few small recent thrombi, but most of the veins are free. There is extreme extravasation of red blood cells into its stroma. The other portion of the anatomical protocol may be omitted, as it has no bearing on the points under consideration.

TABLE IX.

*Human Intestinal Obstruction.*

| Time. | Non-coagulable nitrogen.* | Urea nitrogen.* | Urea. | Remarks. |
|---|---|---|---|---|
| | *mg.* | *mg.* | *per cent* | |
| 2 hrs. before operation. | 145 | 30 | 20 | After infusion of 900 cc. of 1% sodium carbonate and 6% glucose. |
| 18 " after " | 76 | 48 | 63 | After infusion of 400 cc. of 6% glucose. |
| 26 " " " | 80 | 52 | 60 | " " " 750 " " 6% " |
| 46 " " " | 164 | 107 | 65 | Heart blood (see Table VII). |

* Non-coagulable and urea nitrogen are given in terms of mg. per 100 cc. of blood.

The human case (Table IX) is of much interest as the observations on the blood are fairly complete, and autopsy material is available. Clinically it was a clear case of obstruction due to volvulus with partial infarct formation in the intestine and a complicating pneumonia. The non-coagulable nitrogen of the blood was constantly high in spite of transfusion at various times. The elimination of urine was considerable, because of the transfusions, and showed nothing of interest—a mere trace of albumin and an occasional cast.

Table IX shows that there is a rise in urea and drop in non-coagulable nitrogen after the first infusion. We have noted this under the same circumstances in dogs, but are not able to advance an explanation. The high creatinine value (Table VIII) is of interest, as the kidneys are shown to be practically normal. This case gives blood findings identical with those observed in animals under similar conditions, and indicates that our experiments give information which may be of value in the study of human cases.

## DISCUSSION.

We believe that it may be assumed as proven that the non-coagulable nitrogen of the blood in the majority of instances is definitely

increased in intestinal obstruction or with closed loops of intestine. With acute intoxication this rise in non-coagulable nitrogen is apt to be more striking and constant. When this rise in non-coagulable nitrogen of the blood does occur, it is a grave sign, and is a clinical index of a severe intoxication even in spite of other clinical evidence to the contrary. But a low non-coagulable nitrogen does not guarantee a mild grade of intoxication. We are convinced that a knowledge of the blood non-coagulable nitrogen is of considerable clinical value in the prognosis of acute abdominal conditions.

It should be kept in mind that the urea nitrogen as well as creatinine nitrogen may show high values in these conditions, and these points may be of value in differential diagnosis. It is established that other conditions besides chronic nephritis may show a marked increase in the creatinine and urea nitrogen of the blood. It should be recalled that "creatinine rises above 2.5 mg. per 100 cc. of blood almost without exception only in conditions with renal involvement" (Myers and Lough (6)). The conditions studied by us show a high creatinine fraction and constitute exceptions to this statement.

It will be noted that the undetermined nitrogen in these experiments is unusually high—more so than in cases of nephritis with high non-coagulable nitrogen. This may be a peculiarity of this type of intoxication as contrasted with simple retention of nitrogenous material, and a study of this point may bring out much valuable information.

Having established the fact that the non-coagulable nitrogen of the blood is much increased in many cases of intestinal obstruction or of closed intestinal loops, we may now ask: Why does not the kidney eliminate these substances immediately? The kidneys are normal in gross and by functional tests in practically all cases. There can be no true retention because of impaired kidney function. There may be two or more factors concerned. We know that in intestinal obstruction the current of fluid is mainly out of the body and by way of the intestinal tract, and it is possible that this favors the accumulation of certain products in the blood stream. The kidneys excrete small amounts of highly concentrated urine.

It is to be remembered, too, that injection of a small amount of a toxic proteose may cause a great rise in non-coagulable nitrogen in

the blood; for example, a rise from 40 to 80 mg. in 3 hours. This cannot be due to lack of elimination, and we must assume destruction of body protein to account for this remarkable change. We may assume that any acute proteose intoxication may be associated with a similar rapid rise in non-coagulable nitrogen in the blood. When we have more information about this point, we may better understand the manner in which the toxic proteoses injure the body and perhaps the various methods of body defense.

General peritonitis is often associated with a definite rise in non-coagulable nitrogen of the blood. How may we explain this observation? It may be argued that paralytic ileus is alone responsible, and this may be true in part. However, we think it important that a toxic proteose can be isolated from the exudate in cases of general peritonitis, and obviously must be absorbed by the host. The proteose intoxication may well be responsible for this change in non-coagulable nitrogen. We hope to report further on this point in the near future.

## SUMMARY.

Intestinal obstruction, as a rule, is associated with an increasing amount of non-coagulable nitrogen in the blood. With acute intoxication the rise in non-coagulable nitrogen may be rapid and reach as high as three or even ten times normal. With more chronic intoxication there may be little or no rise in the blood non-coagulable nitrogen.

Closed intestinal loops show exactly the same picture, and, when combined with obstruction, may give very high nitrogen readings.

Acute proteose intoxication due to injection of a pure proteose will show a prompt rise in blood non-coagulable nitrogen, even an increase of 100 per cent within 3 or 4 hours.

These intoxications also show a high blood content of creatinine and urea. The residual or undetermined nitrogen may be very high.

A human case of intestinal obstruction with autopsy presents blood findings exactly similar to those observed in many animal experiments.

Clinically the non-coagulable nitrogen of the blood may give information of value in intestinal obstruction. A high reading means

a grave intoxication, but a low reading may be observed in some fatal cases and gives no assurance that a fatal intoxication may not supervene.

The kidneys in practically all these experiments are normal in all respects.

It is possible that protein or tissue destruction rather than impaired eliminative function is responsible for the rise in non-coagulable nitrogen of the blood in these acute intoxications.

Transfusions of dextrose solutions often benefit intestinal obstruction, and may depress the level of the non-coagulable nitrogen in the blood. Some cases show no change in non-coagulable nitrogen following transfusions and diuresis, and, as a rule, such cases present the most severe intoxication.

The writers wish to express their appreciation of the assistance given by Dr. A. R. Kilgore and Dr. T. Stearns of the University of California Hospital.

BIBLIOGRAPHY.

1. Tileston, W., and Comfort, C. W., Jr., *Arch. Int. Med.*, 1914, xiv, 620.
2. Folin, O., *Jour. Biol. Chem.*, 1914, xvii, 475.
3. Van Slyke, D. D., and Cullen, G. E., *Jour. Biol. Chem.*, 1914, xix, 211.
4. Benedict, S. R., *Jour. Biol. Chem.*, 1915, xx, 629.
5. Whipple, G. H., Rodenbaugh, F. H., and Kilgore, A. R., *Jour. Exper. Med.*, 1916, xxiii, 123.
6. Myers, V. C., and Lough, W. G., *Arch. Int. Med.*, 1915, xvi, 536.

# THE ORIGIN AND STRUCTURE OF A FIBROUS TISSUE FORMED IN WOUND HEALING.

By GEORGE A. BAITSELL, Ph.D.

(*From the Osborn Zoological Laboratory of Yale University, New Haven.*)

Plates 106 to 109.

(Received for publication, March 14, 1916.)

## INTRODUCTION.

In a previous paper[1] the author showed that in living cultures of adult frog tissues there occurs, in many instances, a transformation of the plasma clot in which the living tissue is imbedded. This transformation results in a consolidation or fusion of the elements of the fibrin net and a consequent formation from it of a fibrous tissue which is identical in its form and structure and in many of its staining reactions with a regular collagenous connective tissue. It was felt that, whether or not this new fibrous tissue directly formed from the fibrin clot remained as a permanent connective tissue, such a reaction must play a fundamental part in the processes attendant upon wound healing. The present paper[2] gives the results obtained from an extensive series of experiments undertaken for the purpose of studying the action and fate of the fibrin clot formed during wound healing, and they give evidence that in the healing of skin wounds in the frog a definite transformation of the fibrin clot takes place such as was found to occur in the tissue cultures. This transformation also results in the formation of a new fibrous tissue, without intracellular action, which is apparently identical with regular permanent connective tissue.

[1] Baitsell, G. A., The Origin and Structure of a Fibrous Tissue Which Appears in Living Cultures of Adult Frog Tissues, *Jour. Exper. Med.*, 1915, xxi, 455.

[2] A report of this work was given at the meeting of the American Association of Anatomists held at New Haven, Conn., Dec. 28–30, 1915. An abstract appears in the proceedings in the *Anat. Rec.*, 1916, x, 175.

*Material and Methods.*

It was thought best to carry on the experiments in wound healing with the frog inasmuch as the tissues used in the previous work with living tissue cultures had been obtained from this animal. During the present experiments thirty-one animals were used and a total of fifty operations were performed. The process of wound healing has been studied at various stages both in the living animals and in the preserved material. For the study of the preserved material pieces of the tissues containing the wounds were removed at various times after the operations. These were preserved in Zenker's solution, imbedded in paraffin, and sections, in various planes, were made at 10 $\mu$. In general the stain used has been Mallory's connective tissue stain modified according to Mall.[3] Mallory's stain, unmodified, and the Van Gieson picro-fuchsin stain have also been used for comparison. A discussion of the staining reactions will be found in a later section of this paper.

In the preliminary experiments, the semimembranosus muscle lying on the dorsal surface of the hind legs of the frog was used. After anesthetization the animal was placed on an operating board dorsal surface up. The skin was then sterilized by washing it with a 0.001 per cent solution of mercuric chloride. This method of sterilization appears to be adequate for this work and there has been very little trouble from infection. An incision of about 5 to 7 mm. in length was then made in the median dorsal surface of either or both of the hind legs. The cut edges of the skin were separated and held apart and a cube of the underlying muscle tissue measuring about 2 mm. was removed. The cavity thus formed in the muscle tissue was either filled with plasma which had been obtained in the usual manner[4] from another frog, or by the blood and lymph which flowed into the wound from the cut edges. After the operation the frog was held in position for a few minutes until the clot had formed in the wound.

The results obtained from these experiments were not satisfactory. The many movements of the legs and the consequent expansion and

[3] Mall, F. P., On the Development of the Connective Tissues from the Connective-Tissue Syncytium, *Am. Jour. Anat.*, 1901–02, i, 338.

[4] Baitsell, G. A., The Origin and Structure of a Fibrous Tissue Which Appears in Living Cultures of Adult Frog Tissues, *Jour. Exper. Med.*, 1915, xxi, 456.

contraction of the muscle fibers resulted, in many cases, in a breaking up of the plasma clot in the wound cavity and its destruction, so that it was impossible to make a study of it. The friction of the overlying skin of the leg during these movements also tended to make the operations unsuitable for the present study of the fibrin clot. In a number of these experiments which were first made, stitches were taken in the skin to hold the cut edges together and in their proper positions. In the later operations it was found that this was not necessary for it was observed that when a cut was made in the skin, as noted above, if the animal remained quiet for a few minutes after the operation, the coagulation of the blood and lymph formed a tissue of sufficient strength to hold the cut edges of the skin in their proper position. It appeared evident from this that wounds in the frog skin would afford a suitable field for the study of the process of wound healing, particularly with regard to the action and fate of the fibrin clot. Later experiments showed that the skin lying on the posterior dorsal surface of the animal offered an even better field for these experiments than did the skin of the legs inasmuch as it is not subject to an almost continual, and at times violent strain as the result of leg movements. Wounds in both these regions have been studied and form the basis of the results given in the present paper.

### *Observations on the Process of Wound Healing in the Skin of Living Frogs.*

The healing of various types of wounds has been observed in the living animals. In the first type of experiments, a simple incision was made in the skin of the frog either on the dorsal surface of the leg or on the back. As far as could be told by the observations on the living animal these wounds healed very quickly. The formation of the coagulation tissue as a result of the clotting of the blood plasma or lymph present in the wound occurred within a few minutes after the wound was made. As a result the cut edges were firmly held in position and the frog could then be placed in the aquarium without injury to the new tissue. The animals examined 24 hours later showed only slight evidence of the wound. The cut edges were still firmly held and all that could be noted was the light colored line of coagu-

lation tissue marking the extent of the incision. In the course of the next day or so the epithelial cells moved over this area and obliterated all evidences of the wound.

In the second type of experiments a piece of skin measuring about 5 by 2 mm. was detached on three sides. The fourth side (2 mm.) was left attached to the body. In this way a flap of skin was formed which was severed from the body except at one end. In such cases the coagulation tissue which quickly formed held all the cut edges of the skin and the loose flap in place, and thus an operation which one would naturally expect, without the aid of sutures to hold the edges in place, to have left a large open wound in the skin with a consequent slow healing was permanently closed within a few minutes after the operation. In the following days one could observe, as in the previous type of experiments, a gradual obliteration of the cuts by the epithelial cells which moved out over the coagulation tissue from the cut edges of the skin.

In the third type of experiments a piece of skin from the median posterior region of the back, measuring about 5 mm. square, was completely severed from the body of the animal, placed in sterile Ringer solution for a few minutes, and then replaced in the cavity from which it had previously been taken. The observations showed that no other support than the coagulation tissue which formed was necessary in order to hold such a piece of skin in position in the wound cavity, and the complete obliteration of the wound occurred in a few days by the movement of the epithelial cells over the coagulation tissue. The results of other experiments of this type showed that the living skin from another animal or a dead tissue, such as the stratum compactum obtained by pancreatin digestion of adult frog skin, could be transplanted into a wound cavity in the skin and retained there by the action of the coagulation tissue alone. In a later paper it is hoped that more complete results regarding such skin transplantation can be presented. The only fact regarding them which should be emphasized in the present paper is the action of the coagulation tissue in the wound cavities as shown by its ability to hold the transplant in position without the aid of sutures.

In the last type of experiments, a piece of skin, varying in size from 3 to 5 mm. square, was completely removed from the body. The resulting cavity was quickly filled with the coagulation tissue. In this

type of experiment it was found that in some cases, particularly if the cavity caused by the removal of a piece of skin was large, it was better to supply additional plasma obtained from another animal in order that the wound cavity might be completely filled. A wound of this type (Fig. 1) gives the best opportunity for the study of the coagulation tissue. It forms a hyaline mass in the wound cavity and is glossy and transparent, and until it has been covered by the epithelial cells which move in from the surrounding areas the underlying muscle tissue can be seen through it. This fibrin clot or coagulation tissue when it is first formed may sometimes be destroyed by extremely violent movements of the animal, especially if the wound be a large one. An increase in the strength of the new tissue soon becomes evident and in a few days it appears to be as immune from injury as regular skin tissue. As far as can be told by the observations on the living animals, this coagulation tissue formed by the clotting of the plasma in the wound remains permanently and takes the place of the piece of skin tissue which was removed. From this it is clear that in a skin wound in the frog, if the conditions are right, a new tissue can be formed almost immediately after the injury which is sufficiently strong to retain its place in the wound cavity and to hold the cut edges in position. The wound, to all appearances, is thus healed without waiting for a process of regeneration of tissues by cell division. Probably the most impressive part of this process of wound healing, to an observer, is the rapidity with which it takes place. The coagulation of the plasma closes the wound almost immediately and then, evidently by a transformation of this fibrin clot, a firm, resistant, and apparently permanent tissue is formed which serves as a regular connective tissue.

During the course of the experiments, observations have also been made on the healing of wounds in which no coagulation tissue was present. Such a condition may arise either because of the lack of a sufficient amount of plasma in the wound at the time it was made, because of a later destruction of the coagulation tissue as a result of tension caused by too vigorous movements, or because of an infection. In cases of this kind the process of wound healing in the skin of a frog is a comparatively long and slow one, and in some cases results in the death of the animal by a later infection received through the open wound.

*Study of the Prepared Material.*

In Fig. 1 a view of a wound in the skin of a frog is shown *in toto* at a magnification of about 15 diameters. This wound was removed from the animal and preserved when it was three days old. The wound, caused by the removal of a piece of skin, originally measured about 5 by 2 mm. The figure was drawn with the aid of a Zeiss binocular microscope from the under surface of the skin and shows the wound surrounded by the skin tissue and filled with the coagulation tissue (fibrin clot) which was formed within a few minutes after the wound was made by a clotting of the blood plasma and lymph. Several large blood vessels are shown, some of which run into the coagulation tissue. Some of these vessels appear to be enlargements of the vessels supplying the skin in that region, while others apparently arise in the underlying muscles. In some cases the wound is found to be attached to the muscles by a number of small vessels.

A transverse section of the same preparation is shown in Fig. 2 at a magnification of 77 diameters. At either end of this figure may be noted the cut ends of the skin which mark the boundaries of the wound. Connecting these can be seen the bridge of new tissue which has formed in the wound cavity. Lying above this coagulation tissue are several layers of epithelial cells which have wandered in from the skin tissue surrounding the wound. This movement of the epithelial cells begins very soon after the wound has been made and continues until, as shown in later stages (Fig. 6), they form a thick layer over the coagulation tissue. The epithelial cells in Fig. 2 are more numerous near the cut edges of the skin. At these points the coagulation tissue is drawn out into a rather thin strand which is attached to the cut edges of the skin and which serves to hold them in place. It can be noted in this figure that fibers are present in the coagulation tissue.

The observations on the living animals have shown that the coagulation tissue in a 3 day wound is very firm and resistant as compared with a young fibrin clot. From this it is evident that already some change has taken place in the clot and this fact can also be noted from the study of the prepared material. In Fig. 3 a portion of the coagulation tissue, from the same preparation as Fig. 2, is shown at

a magnification of 787 diameters. At this magnification one is able to see clearly the histological structure of the coagulation tissue. Quite large areas can be noted in this figure in which the fibrin net has not been changed but still retains its characteristic structure. In other regions, however, the fibrin net has been changed into a new fibrous tissue. In such areas it appears that there has been a fusion of the elements of the fibrin net to form long, wavy, fibrous bundles which are typical in structure and appearance to those found in various forms of connective tissues. This new fibrous tissue is apparently identical with that which forms in plasma clots in tissue cultures. The transformation of the fibrin clot in this wound into a new fibrous tissue has not been due to an intracellular action for, with the exception of some blood corpuscles which were present in the plasma when it clotted, some of which can be seen in Fig. 3, no cells of any kind are present in the new fibrous tissue except in the extreme edges where the new tissue is in contact with the cut edges of the skin. A thorough study of this point has been made in this and other similar preparations and it can be definitely stated that in wounds in the frog skin, as has previously been shown to be the case in tissue cultures, a direct transformation of a fibrin clot into a new fibrous tissue will occur without intracellular action.

In Fig. 4, which is a portion of a longitudinal section of a 4 day wound at a magnification of 787 diameters, a more complete transformation of the plasma clot is seen. The section from which this figure was drawn was taken from near the upper surface of the coagulation tissue; that is, from the dorsal portion of the clot lying just below the epithelial cells. At either edge of the coagulation tissue may be noted some of the epithelial cells which mark the boundary of the wound and which have grown down on both sides of the coagulation tissue. This downward growth of the epithelial cells can be noted in some of the transverse sections of the wound tissue (Fig. 2). A study of Fig. 4 shows that the fibrin net structure has disappeared and the new fibrous tissue has been formed which, as in Fig. 3, is composed of bands of fibrils showing the typical structure of regular connective tissue fibers. The new fibrous tissue is denser near the bottom of the figure and it is in this region that its structure is seen to the best advantage. All the coagulation tissue shown in

the figure has lost the original fibrin net structure, but in the less dense regions of this tissue, in which numerous vacuoles are present, the transformation into the new fibrous tissue has not been so complete. No fibroblast cells are present in this region of the clot and so in this case also the transformation of the fibrin clot into the new fibrous tissue has not been due to any intracellular action. In this preparation centrifuged blood plasma which had been obtained from another animal was placed in the wound to form the clot and this fact accounts for the almost complete absence of blood corpuscles. Those that are shown in this figure are in most cases matted together and are evidently undergoing degeneration.

In Fig. 5 is shown, at the same magnification as Fig. 4, another longitudinal section of the same preparation taken at a deeper level. In this figure a considerable number of both fibroblast cells and blood corpuscles are shown. In this figure a transformation of the fibrin clot has also occurred and a new fibrous tissue has been formed containing bundles of wavy fibers. These are particularly well shown to the left of the figure. In this region numerous cells are also present and as a result the bundles of fibers are more or less separated from each other. To the right of the figure fewer cells are present and the newly formed fibrous tissue is more closely massed together. The bundles of fibers, however, can be clearly seen.

A comparison of the new fibrous tissue shown in Figs. 3, 4, and 5, which has been formed through a transformation of the fibrin clot, with the fibrous tissue found in the plasma clot in living cultures of frog tissues, as noted in a previous paper,[5] shows that they are identical.

The conditions present in an older wound are shown in Fig. 6, which is a transverse section of a 12 to 13 day preparation at a magnification of 240 diameters. At either end of the figure can be seen the cut ends of the skin tissue which mark the boundaries of the wound and between these the new tissue which has been formed in the wound. Overlying this region are the epithelial cells which are piled up many layers thick. The wound cavity, except for a small portion

[5] Baitsell, G. A., The Origin and Structure of a Fibrous Tissue Which Appears in Living Cultures of Adult Frog Tissues, *Jour. Exper. Med.*, 1915, xxi, 478–79. Compare Fig. 3 of this paper with Figs. 11 and 17 of previous paper.

lying near the right end of the figure, is filled with the new fibrous tissue. Fibroblast cells are present all through the new tissue. In the region lying to the right a portion of the original fibrin clot apparently still remains. In Fig. 7, which was drawn from the same section as Fig. 6, this region of the wound is shown at a magnification of 787 diameters. The figure shows that the fibrin clot has lost its original structure, as shown for example in Fig. 3, and has become a more or less compact mass in which, at various places, it can be noted that fibers have formed. Around the edges of the fibrin mass, in particular, a great many of the bundles of fibers can be seen. These are attached to the fibrin clot and it is clear that they have arisen by a transformation of its elements and not from an intracellular action. The cells which are present stand out distinctly from the fibers, as shown in the figure, and show no connection with them.

In Fig. 8 is shown, at a magnification of 787 diameters, a longitudinal section of another wound which is practically the same age as the one shown in Figs. 6 and 7. In Fig. 8, however, we find that the formation of the new tissue has proceeded more rapidly and consequently the original fibrin net has been entirely transformed into the new fibrous tissue which fills the wound cavity. Blood vessels are forming in various regions in this new tissue and numerous cells, which for the most part show the typical spindle shape of fibroblast cells, are present but stand out clearly and distinctly from the fibers. The question of the relations existing between the new fibrous tissue and the cells which wander into it from the surrounding edges of the skin is one which has been studied with care. It has already been pointed out that the transformation of the fibrin clot occurs previous to the appearance of any cells, so that it cannot be due to an intracellular action (Figs. 3 and 4). A study of the preparations shows that the cells when they first appear in the new fibrous tissue are round in shape (Figs. 5 and 7). Later they elongate and assume the typical spindle shape of fibroblast cells (Fig. 8). This change in the shape is apparently due to the stereotropic action of the cells in moving along the fibers.[6] The movement of the cells in the newly formed fibrous

[6] Harrison, R. G., The Reaction of Embryonic Cells to Solid Structures, *Jour. Exper. Zool.*, 1914, xvii, 521.

tissue appears to result in a separation of the bundles of fibers and we have as a result what, to all appearances, is a typical scar tissue. With the Mallory stain the cells are colored yellow and they are distinctly differentiated from the fibers of the new tissue which are colored blue by the stain. The preparations do not show any evidence of a digestion by the cells of the new fibrous tissue or of any attempt by them to form new fibers intracellularly. The condition shown in Fig. 8 is typical of the condition found in much older wounds (3 to 4 weeks) and the evidence appears clear from these results that, in the healing of skin wounds in the frog, the new fibrous tissue present in the wound which has been formed by a direct transformation of the fibrin clot remains as the permanent connective tissue.

### *Nature of the New Fibrous Tissue.*

The appearance, structure, and staining reaction with Mallory's stain of the new fibrous tissue are, as shown in the previous paper, identical with those of regularly formed connective tissues. In an endeavor to settle definitely the real relationship existing between regular frog connective tissue and the new fibrous tissue several series of tests have been made.

*Staining Reactions of the Fibrous Tissue Formed in Wounds.*—The Mallory connective tissue stain, either modified or unmodified, has proved to be more specific in its reaction than any other stain that has thus far been used in the work, although various other methods have been tried. This stain will color connective tissue fibers of the frog an almost perfect ultramarine blue if they are not too closely packed together. If, for example, a piece of skin from an adult frog is stained with Mallory's stain it will be found, in general, that the stratum spongiosum, in which the bundles of connective tissue fibers are loosely packed together and in which the individual fibrils can be distinguished, will be colored an ultramarine blue. On the other hand, in the stratum compactum of the same preparation in which the bundles of fibers are closely massed, it will be found that this layer will be stained a color varying from yellow to bright red, depending upon the manipulation of the stain.

The new fibrous tissue formed in the clots shows the same color re-

action to a Mallory stain as does the connective tissue in the skin. When the bundles of fibers in the new fibrous tissue are not closely matted together, as shown for example in Figs. 3, 4, and 5, the Mallory stain gives them a typical blue color as in the stratum spongiosum of the skin. On the other hand, when the new fibrous tissue is very compact and the individual fibrils composing the bundles cannot be distinguished, then, in many cases, the result will be a color varying from yellow to red, similar to that found in the stratum compactum of the skin. The same color also generally results from the Mallory stain in very young preparations in which the clot has not been changed into the new fibrous tissue but retains its original structure.

With a Van Gieson picro-fuchsin stain the new fibrous tissue shows a negative reaction. This stain, however, does not appear to be a specific one for connective tissues of the frog. The results show that it acts in just the opposite way that Mallory's stain does, in that it only gives the characteristic red color for connective tissues when a heavy tissue is present in which the bundles of fibers are closely massed together. In the frog skin, for example, the stratum compactum occasionally stains red, whereas in the stratum spongiosum an entirely negative reaction is obtained. In most cases, however, all the connective tissues of the frog skin as well as those from other parts of the body give a negative color reaction with this stain. In this connection a series of staining tests were made with embryonic connective tissue from the tail of a 50 mm. tadpole. It consists of a mass of connective tissue fibers loosely packed together. These fibers will take a typical stain with Mallory's stain, but with Van Gieson's the reaction is negative, as it is with the new fibrous tissue. The results in brief from all these experiments show that the Van Gieson's stain is not specific for connective tissue fibers in the frog whether embryonic or adult, and the same is true for its reaction with the new fibrous tissue.

*Digestion Tests of the Fibrous Tissue Formed in Wounds.*—Of all the tests used to distinguish between fibrin and connective tissues, the digestion tests are regarded as being the most specific and conclusive. A young fibrin clot is easily digested in a pancreatin solution whereas connective tissues resist the action. In the previous paper it was noted that the transformed fibrin net in the tissue cultures was easily dissolved in

pancreatin, and this fact gave evidence that the new fibrous tissue was fibrin in character although greatly changed in appearance from the typical fibrin net. However, it was also pointed out that in this case the test was not conclusive, inasmuch as the newly formed fibers in the clot were imbedded in a fibrin net which was not completely transformed, and the digestion of the unchanged fibrin clot by the pancreatin would naturally cause a breaking down and scattering of the fibers which had formed in it, and remained connected with it. In other words, the surrounding unchanged fibrin constituted the sole support of the fibers which had formed from a part of it.[7] This close relation existing between the fibrin clot and the new fibrous tissue also prevents the digestion tests being used in the present experiments to furnish a conclusive answer as to the real nature of the transformed fibrin net tissue in the clots formed in wound healing.

In this connection the results secured by a series of experiments in which the embryonic connective tissues of the tadpole were subjected to pancreatin digestion should be noted. In these experiments pieces of skin, taken from tadpoles ranging in length from 7 mm. to large tadpoles of about 70 mm. just before metamorphosis, were placed in pancreatin solutions of uniform strength[8] and digested over night at 38°C. In all these experiments a complete digestion of the tadpole skin occurred. This same result was obtained when the connective tissue from the tails of tadpoles of various ages was digested in pancreatin solutions. These young fibers, as has been noted, stain typically with Mallory's stain and are apparently fully formed. Nevertheless they can be completely digested in pancreatin in a few hours. These results with embryonic connective tissues are entirely different from those obtained when connective tissues from an adult frog are subjected to the same treatment, for the experiments show that the mature connective tissues in the skin of an adult frog are able to resist the action of the pancreatin and remain as a mass of tissue which, as noted above, could be used in the implantation experiments.

[7] Baitsell, G. A., The Origin and Structure of a Fibrous Tissue Which Appears in Living Cultures of Adult Frog Tissues, *Jour. Exper. Med.*, 1915, xxi, 473.

[8] The pancreatin extract used in all these digestion experiments was obtained by the well known Roberts' method. The author is indebted to Professor F. P. Underhill for his assistance in this matter.

These results demonstrate that the reaction of the embryonic connective tissues of the frog to pancreatin digestion is the opposite of that of adult connective tissue.

The results of both the staining and digestion tests, in brief, show that with these methods one is unable to differentiate between the new fibrous tissue, formed by a transformation of the fibrin clots, and embryonic connective tissues of the tadpole, and that the connective tissues of the adult, while reacting in the same way to the stains, can be differentiated from both the new fibrous tissue and embryonic connective tissue by their ability to resist the pancreatin digestion.

There is the possibility that the same resistance to pancreatin digestion which must develop in embryonic connective tissue as it becomes mature, as shown by the digestion tests on the mature and embryonic connective tissues, might also develop in time in the new fibrous tissue which has resulted through a transformation of the fibrin clot, for it is well known that old fibrin clots become highly resistant to various agents which will entirely dissolve younger clots. Mall[9] says: "Fresh fibrin is easily dissolved in dilute acid or digested in pancreatin. After fibrin becomes older, as in fibrinous deposits, the fibrils become very resistant and are not dissolved in boiling acetic acid, 20 per cent, in cold concentrated KOH, $H_2SO_4$, HCl, $HNO_3$, or nitro-hydrochloric acid." What changes take place in these fibrinous deposits that cause such an increase in their resisting powers is apparently not known. From the present results one would naturally suggest that in such cases, in the course of time, a complete transformation of the fibrin clot into the new fibrous tissue had occurred and that this transformed tissue, when old enough, was able to resist the action of pancreatin and various other destructive agents.

This point has proven to be one which is difficult of solution. The fact that even the fully formed connective tissue of large tadpoles is unable to resist the pancreatin digestion makes it impossible to expect that one could ever keep tissue cultures alive long enough to secure a new fibrous tissue from the fibrin clot which would be able to

[9] Mall, F. P., Reticulated Tissue, and Its Relation to the Connective Tissue Fibrils, *Johns Hopkins Hosp. Rep.*, 1896, i, 182, 194.

resist the pancreatin digestion and thus show definitely that it could not be distinguished from the regular connective tissues in this regard. In wounds the problem is complicated by the presence of the cells which wander into them and through the new fibrous tissue soon after it has been formed from the fibrin clot. The preparations, as stated above, do not show any evidence of an attempt by these cells to form new fibers intracellularly, but, nevertheless, their presence in the wound for the long period of time which evidently is necessary for the new fibrous tissue to become resistant, makes the problem a difficult one.

## DISCUSSION.

The experiments reported in the present paper demonstrate that in wounds made in the skin of adult frogs there occurs, as has previously been shown to be the case in living cultures of adult frog tissues, a direct transformation of the fibrin clot into a new fibrous tissue without any intracellular action. This newly formed fibrous tissue which fills the wound space is apparently identical in appearance, structure, function, and staining reactions with regularly formed permanent connective tissue. It differs from adult connective tissue in the skin of the frog in its reaction with pancreatin digestion. However, this test, as well as all others that have so far been tried, fails to differentiate between the new fibrous tissue and young connective tissue found in tadpoles of various stages.

Two views might be held with reference to the fate of this new fibrous tissue directly formed from the fibrin clot. The first is that it is only a temporary tissue and that it will be replaced in time by a permanent connective tissue formed through an intracellular action of the fibroblasts. This is in accord with the generally prevailing views[10] held at present, which are that the fibrin, in wounds healing by first intention, forms a temporary tissue which holds the wound surfaces in place and stops up the wound thus preventing infection. Later the fibrin disappears, not by liquefaction, but through an actual consumption by the new tissue cells which show a positive chemotropism to the fibrin mass and are thus attracted into the fibrin clot

[10] For a general discussion and summary see Marchand, F., Der Process der Wundheilung, Stuttgart, 1901, 52–55.

in large numbers. The permanent tissues which go to fill the wound space are then formed by these cells through an intracellular action.

The other view is that this new fibrous tissue remains in the wound as a permanent connective tissue. That is to say, at least in wound healing in the frog skin, a reaction occurs which is able to transform a fibrin clot by a direct intercellular action into a permanent fibrous connective tissue. This view is supported by the work of Hertzler[11] who finds that, in experimental peritoneal adhesions in the dog, a new fibrous tissue, which he believes remains permanently, is formed by a transformation of a fibrin net. Speaking of the formation of this fibrous tissue, he says:[12] "My researches have convinced me that the cell is not primarily the active agent, but that the initial processes are chemical and are identical with those of blood coagulation, the cell playing an entirely secondary rôle."

The results obtained in the present experiments also give evidence in favor of the latter view. The fibroblast cells which wander into the new fibrous tissue apparently do not digest the fibers which have previously been formed, nor is there any evidence revealed, by a thorough study of the preparations, of an attempt by these cells to form new fibers intracellularly. Their only apparent action is to break up the larger bundles of fibers by their movements among them. In a wound of about 12 days a typical scar tissue is generally present (Fig. 8), and later stages (3 to 4 weeks) do not show any further changes in the structure of the connective tissue present in the wound.

## SUMMARY.

1. In experimental wounds, made by removing various sized pieces of skin from the frog, there is a rapid coagulation of the blood plasma and lymph to form a coagulation tissue which fills the wound cavity.

2. The observations on the living animals show that the coagulation

[11] Hertzler, A. E., Pseudoperitoneum, Varicosity of the Peritoneum and Sclerosis of the Mesentery. With a Preliminary Note on the Development of Fibrous Tissue, *Jour. Am. Med. Assn.*, 1910, liv, 351; The Development of Fibrous Tissues in Peritoneal Adhesions, *Anat. Rec.*, 1915, ix, 83.

[12] Hertzler, A. E., Pseudoperitoneum, Varicosity of the Peritoneum and Sclerosis of the Mesentery. With a Preliminary Note on the Development of Fibrous Tissue, *Jour. Am. Med. Assn.*, 1910, liv, 352.

tissue becomes more and more resistant and is generally of sufficient strength to hold the cut edges of the wound in place and to retain its position in the wound cavity. It serves, at least temporarily, as a connective tissue and as a base for the epithelial cells which rapidly move in from all the cut edges and cover the wound.

3. The study of the prepared sections of wound tissue show that at first in the coagulation tissue, formed as a result of the clotting of blood and lymph, a typical fibrin net is present in the wound (Fig. 3). Later this fibrin net is transformed into a new fibrous tissue containing bundles of wavy fibers in which, in many instances, the individual fibrils can be noted (Fig. 4). This transformation of the clot and the formation of the new fibrous tissue takes place before the tissue cells wander into the coagulation tissue and therefore cannot be due to an intracellular action. It is a direct transformation of the fibrin clot and is identical with the process which was previously found to take place in the fibrin clots in living cultures of adult frog tissues.

4. The tissue cells, which later move into the new fibrous tissue in large numbers from the surrounding areas, do not digest the fibers but, apparently by their movements, cause a division of the large bundles into smaller ones (Fig. 5). These cells when they first appear in the fibrous tissue are rounded, but later they assume the typical elongated spindle shape of fibroblast cells (Fig. 8). The preparations do not show any connection between these spindle-shaped cells and the fibers which had already formed, nor is there any evidence of a later attempt by them to form new fibers intracellularly.

5. The staining reactions of the new fibrous tissue appear to be identical with the staining reactions of the connective tissue in frog skin. However, the new tissue can be digested in pancreatin and in this reaction it differs from the connective tissue in the skin of the adult frog. On the other hand, extensive experiments with pancreatin on embryonic but fully formed connective tissue, obtained from the tail and skin of tadpoles of various ages, show that pancreatin will digest it just as it does the newly formed fibrous tissue.

## EXPLANATION OF PLATES.

All the figures, with the exception of Fig. 1, were drawn from prepared material which had been preserved in Zenker's solution, sectioned at 10 $\mu$, and stained with Mallory's connective tissue stain.

### PLATE 106.

FIG. 1. 3 day wound from back of frog, shown *in toto* from the under surface. × 15. A piece of skin measuring about 5 by 2 mm. was removed and the cavity thus formed is shown filled with the coagulation tissue (C.T.). A considerable area of the skin tissue (SK.T.) surrounding the wound is shown, and blood vessels (B.V.) are also present.

FIG. 2. Transverse section of the preparation shown in Fig. 1. × 77. Cut edges of the skin tissue (SK.T.) marking boundaries of the wound are shown at both ends of the figure. These are connected by the coagulation tissue (C.T.) in which fibers (FIB.) can be noted at various places. Epithelial cells (EP.C.) which have moved in from the surrounding areas overlie the coagulation tissue. Section of blood vessel (B.V.) and numerous blood corpuscles (B.C.) are also shown.

### PLATE 107.

FIG. 3. Transverse section showing a portion of the coagulation tissue from the same preparation as Figs. 1 and 2. × 787. The figure shows the structure of the coagulation tissue present in a 3 day wound. Some of this tissue retains the fibrin net (F.N.) structure of the original fibrin clot. Other areas show new fibrous tissue (N.F.T.) which has been formed by a transformation of the elements of the fibrin net. Numerous blood corpuscles (B.C.) are present, but no fibroblast cells.

FIG. 4. Longitudinal section of a 4 day wound in the leg of a frog. × 787. The coagulation tissue has been transformed into the new fibrous tissue (N.F.T.) in which bundles of wavy fibers can be seen. Transformation is most complete in the denser region of coagulation tissue lying to the right of the figure. No fibroblast cells are present, but a few blood corpuscles (B.C.) are shown which are evidently undergoing degeneration.

FIG. 5. Longitudinal section from the preparation shown in Fig. 4. × 787. The section from which this drawing was made was taken at a deeper level of the wound than Fig. 4; *i.e.*, just above the underlying muscle tissue. To the right the new fibrous tissue (N.F.T.) is more dense, but even here the fibrous structure can be seen. To the left it is broken up into wavy fibrous bundles among which numerous cells (FBL.C.) are appearing. Blood corpuscles (B.C.) are also present.

### PLATE 108.

FIG. 6. Transverse section of a 12 to 13 day wound preparation. × 240. Cut ends of the skin tissue (SK.T.) are shown at the right and left of the figure.

The wound cavity is almost filled with the new fibrous tissue (N.F.T.) except for a small portion of fibrin clot (F.C.) lying to the right. Epithelial cells (EP.C.) are piled up many layers thick above the new tissue. Cells (FBL.C.) are also present in the new tissue. Compare Fig. 7.

FIG. 7. A portion of the preparation shown in Fig. 6. × 787. This drawing shows the remains of a fibrin clot (F.C.) lying to the right in Fig. 6. It has lost its original fibrin net structure and has become a more or less compact mass. Numerous fibers can be seen running through it, and around the edges are a great many fibers which have resulted from a transformation of the fibrin clot and which go to form the new fibrous tissue (N.F.T.). Numerous rounded cells (FBL.C.) are present, some of which are beginning to assume the typical spindle shape of fibroblast cells. They stand out clearly and show no connection with the fibers.

## PLATE 109.

FIG. 8. Longitudinal section of another 12 to 13 day wound preparation. × 787. This figure shows complete transformation of coagulation tissue into new fibrous tissue (N.F.T.) which fills the wound cavity between the cut edges of the skin tissue (SK.T.). Blood vessels (B.V.) are forming and numerous spindle-shaped fibroblast cells (FBL.C.) are present. These cells stretch out along the fibers which had previously been formed through a transformation of the fibrin net and apparently separate the bundles. They do not digest the fibers nor is there any evidence of an attempt by them to form new fibers intracellularly.

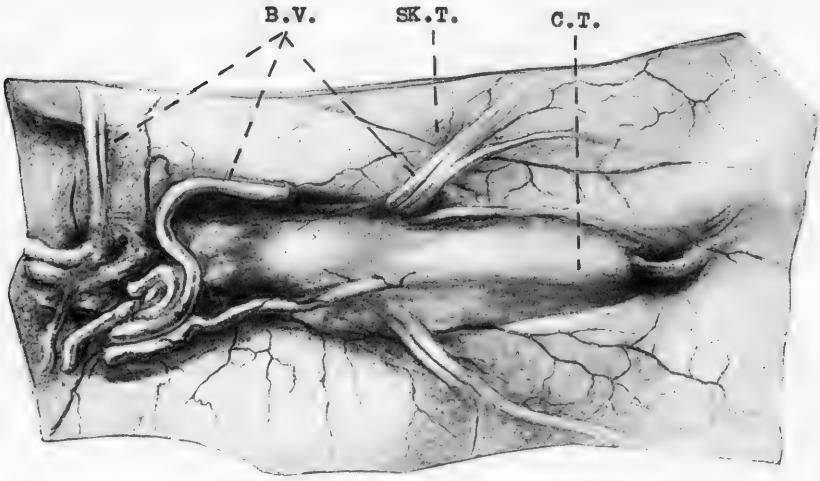



FIG. 1.

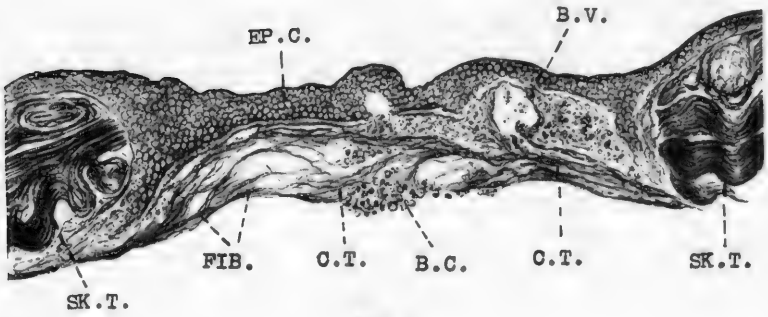



FIG. 2.

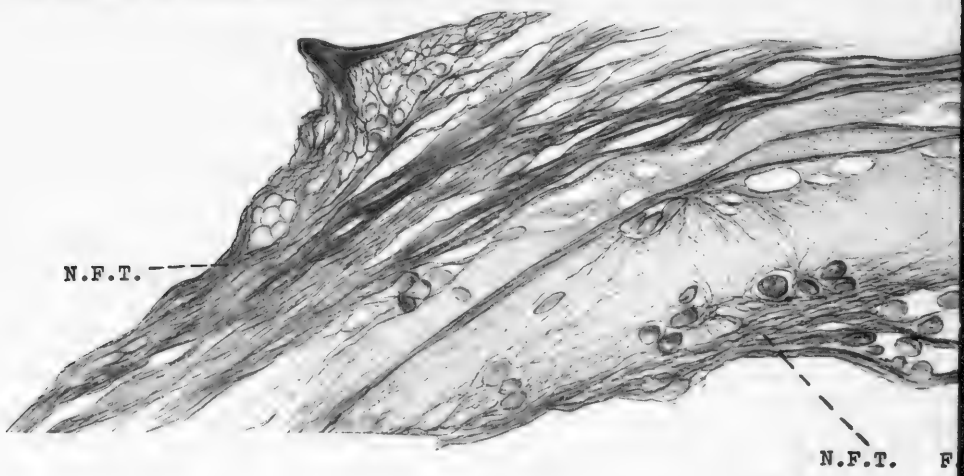



FIG. 3.

FIG. 4.

III.

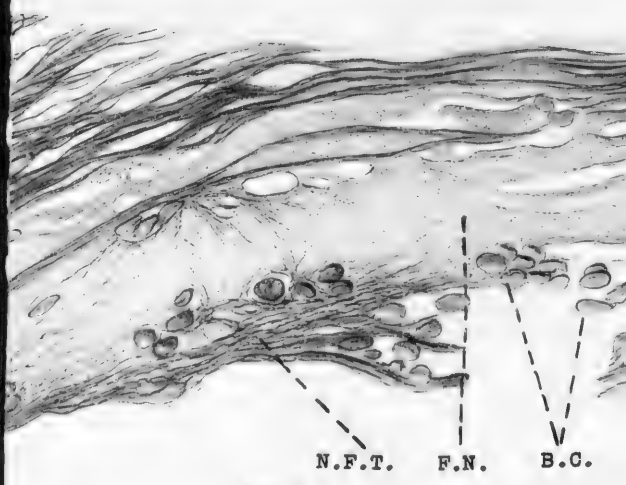
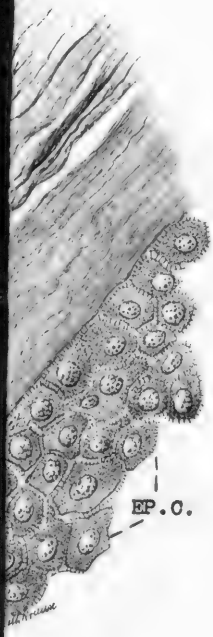
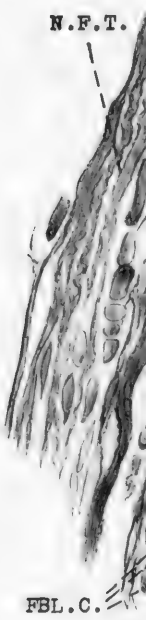



FIG. 3.



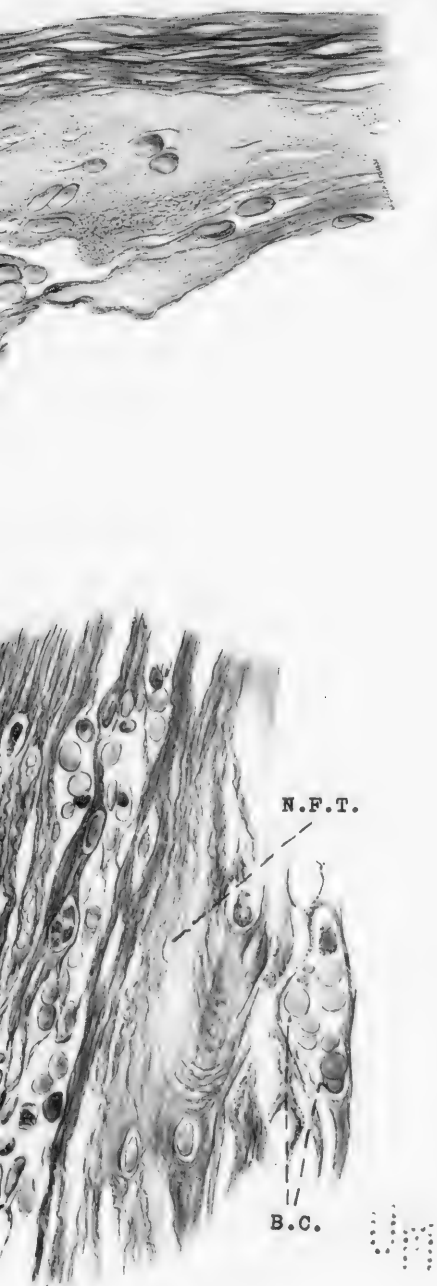



FIG. 5.

(Baitsell: Fibrous Tissue Formed in Wound Healing.)

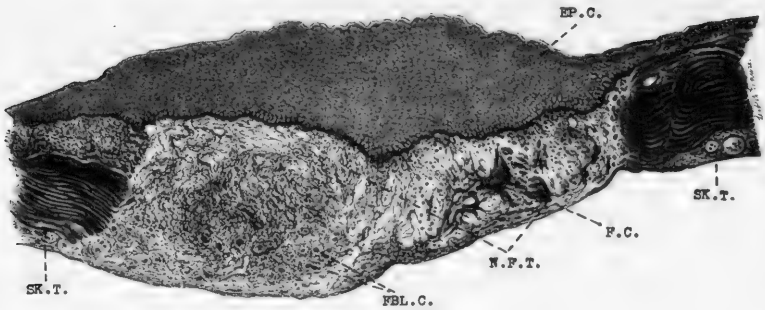



FIG. 6.

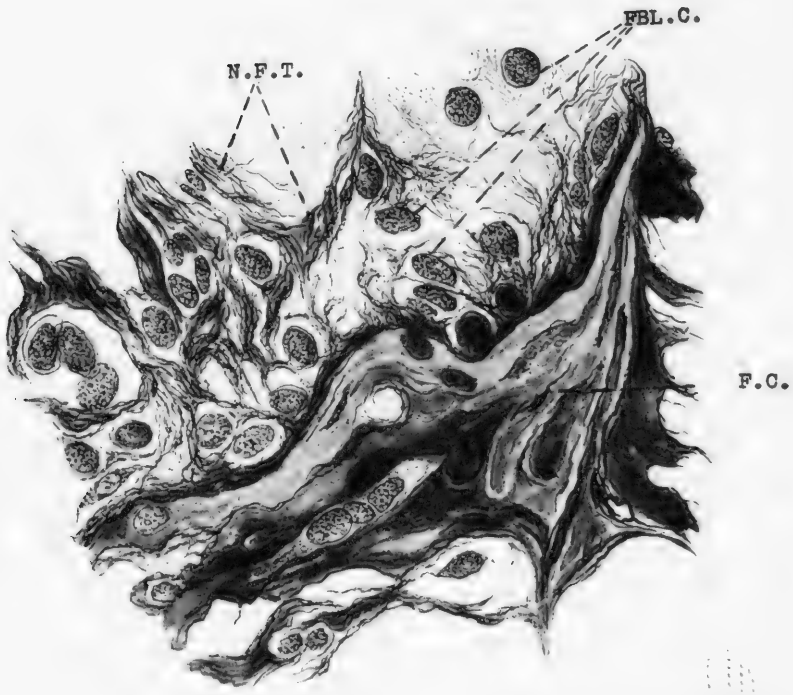



FIG. 7.

(Baitsell: Fibrous Tissue Formed in Wound Healing.)

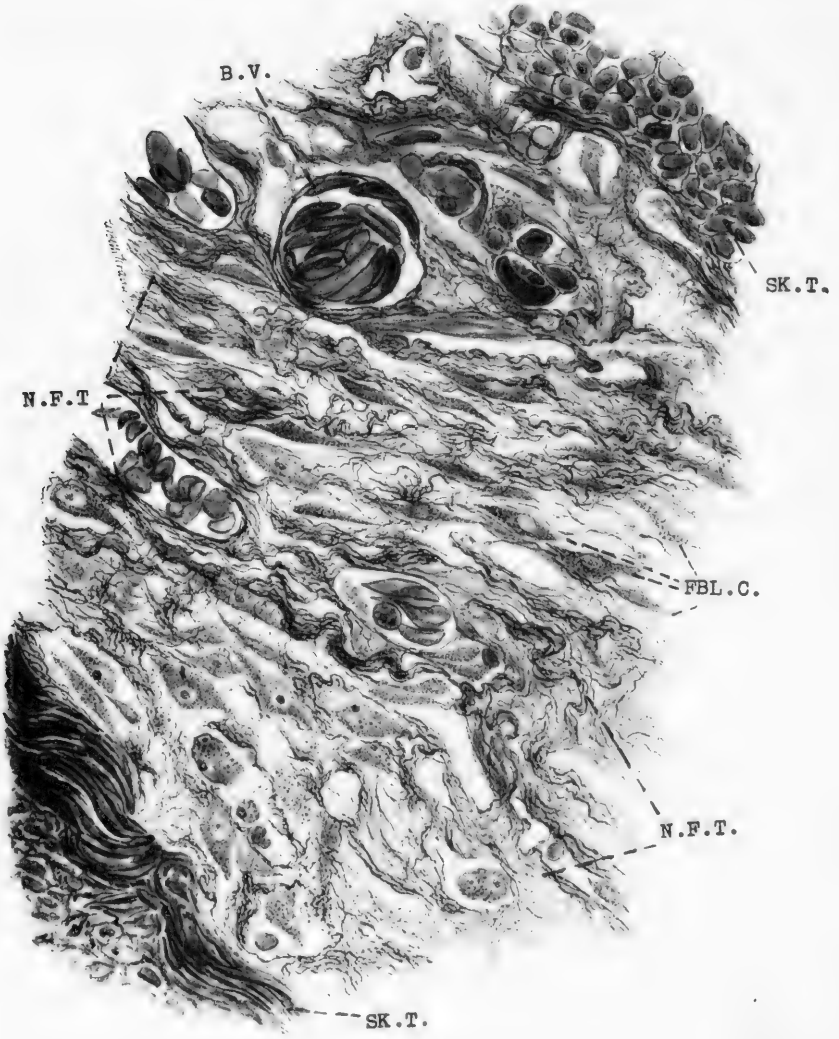



FIG. 8.

(Baitsell: Fibrous Tissue Formed in Wound Healing.)

# THE ABSORPTION OF ADRENALIN AFTER INTRATRACHEAL INJECTION.*

By J. AUER, M.D., and FREDERICK L. GATES, M.D.

(*From the Department of Physiology and Pharmacology of The Rockefeller Institute for Medical Research.*)

(Received for publication, April 11, 1916.)

In order to subject a living organism to the systemic action of any soluble substance, it is obvious that the substance must first reach the circulating fluids of this organism; from the lymph and blood streams the drug then may pass into the tissues and exert its effect. The main routes available for bringing any substance into contact with the tissues are as follows: (1) by introduction into the gastro-intestinal canal; (2) by subcutaneous, intramuscular, intravenous, or intraspinal injection; (3) by inunction through the skin; and (4) through the respiratory tract.

Of these routes the first two are most frequently employed in human therapeutics and in the laboratory. Inunction is used only exceptionally at the present time, and practically the only drug administered through the skin for its systemic action is mercury.

The respiratory route also is not utilized to any extent, except for purely local effects, when it is desired to incorporate a drug. Syphilis was occasionally treated by allowing the patient to inhale the sublimated metal, but this method was never extensively employed because of the impossibility of judging the dose. Local affections of the respiratory passages, for example, laryngitis, bronchitis, and bronchiectasis, are treated by allowing the patient to inhale the vapors of boiling water to which various substances (creosote, eucalyptol, opium preparations, etc.) have been added. Experimentally the respiratory passages are practically not utilized when a drug is to be administered for its general action.

*A preliminary report was published in the Proceedings of the Pharmacological Society (*Jour. Pharm. and Exper. Therap.*, 1914–15, vi, 608).

Among the relatively few experimenters who have used the intratracheal method for this purpose we may mention Külbs,[1] who injected rabbits repeatedly for a number of days with adrenalin, introducing the hypodermic needle into the trachea through the skin of the neck. He observed cough after the injection, and twice an animal died a few minutes after the injection, from pulmonary edema. In those animals which survived the injections, which were given every day or every other day for 22 to 78 days, the total amount being 3.4 to 14 cc. of adrenalin, he found the same macroscopic and microscopic alterations, though of smaller extent, which he obtained after intravenous injection of adrenalin.

Ephraim[2] in a series of papers reports the results of endobronchial treatment of chronic bronchitis and asthma in the human subject. Various drugs, including adrenalin, were introduced as a fine spray into the bronchi, often through a bronchoscope. The effects obtained were chiefly local; even after the bronchial administration of 1 mg. of adrenalin by means of a nebulizer, he obtained no definite rise of blood pressure. In a dog, however, the same method yielded a powerful rise of blood pressure when a large amount, 2 mg., was used.

The failure of Ephraim to obtain a rise of pressure after the administration of 1 mg. of adrenalin endobronchially in the human subject is perhaps attributable to the method. Ephraim himself observed in rabbits that the spray of a colored solution from a nebulizer, even when introduced into the trachea, did not reach the bronchi, the vapor being precipitated near the point of application. In the same way the endobronchial spray of adrenalin was possibly precipitated in the larger bronchi so that only negligible amounts reached the alveolar region where, as we shall show, the absorption is best.

That even colloids can be absorbed with rapidity when injected intratracheally is shown by Ishioka.[3] Ishioka sensitized guinea pigs by the subcutaneous injection of human serum and after 12 to 15 days injected 0.05 to 0.1 cc. of human serum into the trachea of these animals with the object of producing an anaphylactic pneumonia. He observed the milder anaphylactic symptoms in the majority of his experiments, but in two instances acute death with the typical lung picture resulted.

The absorptive capacity of the supratracheal respiratory passages does not concern us here; references concerning the effect of inhaled substances will be found in the papers by Ephraim.

Our own experiments will show that the intratracheal injections of adrenalin are rapidly absorbed even under disadvantageous conditions and exert a systemic effect, and also that this method may be of value therapeutically when a rapid action on the heart is desired.

[1] Külbs, *Arch. f. exper. Path. u. Pharm.*, 1905, liii, 150.

[2] Ephraim, A., *Deutsch. med. Wchnschr.*, 1911, xxxvii, 2079; 1912, xxxviii, 1453.

[3] Ishioka, S., *Deutsch. Arch. f. klin. Med.*, 1912, cvii, 500.

*Methods.*

The research was carried out entirely with rabbits, and the chief test substance employed was adrenalin. Adrenalin was chosen because its absorption could be readily detected by recording the blood pressure, and the character and degree of the resultant rise would give some indication of the speed and amount of absorption.

The animals were anesthetized by ether for the operative interferences. These were the insertion of a cannula into the right carotid artery; transection of the trachea with introduction and ligation of a wide glass tube about 2 cm. long into the distal stump so that the respiratory path was free and injection into the trachea easy; in some rabbits the tracheal cannula was inserted as near the thorax as possible and the lower end of the proximal trachea then ligated, thus converting the cervical trachea into a sac from which the absorption of adrenalin could be tested; in a number of experiments both vagi were sectioned in the neck.

The blood pressure was written by a mercury manometer connected with the artery by tubing filled with a half saturated solution of sodium sulphate.

The adrenalin used was generally the commercial solution in 0.1 per cent strength, preserved with chloretone. In some experiments, however, a solution was made from the commercial powder preparation, and this was used with or without the addition of chloretone.

The dose injected varied from 0.15 to 0.03 cc. per kilo of body weight. The maximum dose quoted was occasionally greatly exceeded when adrenalin was injected intramuscularly.

The solutions were injected into the respiratory passages in several ways. Usually the required amount was injected in 4 to 6 seconds from a Record tuberculin syringe into the tracheal cannula. In a number of experiments a filiform catheter was introduced into a bronchus and the solution then driven in by a gentle blast of air. A few times the solution was injected directly into the lung tissue by passing the hypodermic needle through the walls of the chest.

In a majority of the experiments doses of adrenalin were injected repeatedly, not only into the trachea but also into the erector spinæ muscles of the back.

The rabbits were always placed on an electric warming pad to reduce or prevent the loss of body heat.

## EXPERIMENTAL RESULTS.

Twenty-three experiments were carried out in the adrenalin series; in ten of these tests both vagi were cut previous to the injection of the drug. The course of the experiments and the characteristic effects will be illustrated by a few typical protocols arranged in tabular form.

All rabbits tabulated were tracheotomized under light ether anesthesia; there was no insufflation of air except in No. 3 (Table I); vagi

were intact, except in No. 8 (Table IV); all injections into the trachea were made with a syringe through the tracheal tube.

TABLE I.

| No. of animal. | Color. Sex. Weight. | No. of injection. | Method and site of injection. | Amount of adrenalin per kilo. | Latent period of blood pressure rise. | Blood pressure rise. | Duration of blood pressure rise. | Interval between injections. |
|---|---|---|---|---|---|---|---|---|
| | | | | *cc.* | *sec.* | *mm.* | *min.* | *min.* |
| 3 | | 1 | Catheter into bronchus. | 0.23 | 12 | 64 (104–168) | 8 | |
| | | 2 | Trachea. | 0.23 | — | 45 (90–135) | 9 | 9 |
| | Gray ♀ | 3 | " | 0.23 | 6 | 25 (107–132) | 9 | 10 |
| Vagi intact. | 2,210 gm. | 4 | Erector spinæ muscle. | 0.23 | No rise in 4 min. | | | 10 |
| | | 5 | Erector spinæ muscle. | 0.46 | " " " 4 " | | | 4 |
| | | 6 | Trachea. | 0.23 | 7 | 24 (96–120) | 18 | 4 |
| Killed by medullary puncture after experiment. Lungs showed only a moderate degree of pulmonary edema. | | | | | | | | |
| 6 | | 1 | Tracheal sac. | 0.25 | 52 | 16 (91–107) | 15 | |
| | | 2 | Trachea. | 0.25 | 12 | 60 (100–160) | — | 17 |
| | Gray ♂ | 3 | Tracheal sac. | 0.25 | No rise in 6 min. | | | 16 |
| Vagi intact. | 2,100 gm. | 4 | Trachea. | 0.25 | 3 | 40 (62–102) | 10 | 8 |
| | | 5 | Erector spinæ muscle. | 0.5 | No rise in 7 min. | | | 10 |
| | | 6 | Trachea. | 0.25 | 4 | 26 (80–106) | 7 | 10 |
| Killed later by medullary puncture. The lower lobes of both lungs showed well marked pulmonary edema; slight in upper lobes. | | | | | | | | |

TABLE II.

| No. of animal. | Color. Sex. Weight. | No. of injection. | Method and site of injection. | Amount of adrenalin per kilo. | Latent period of blood pressure rise. | Blood pressure rise. | Duration of blood pressure rise. | Interval between injections. |
|---|---|---|---|---|---|---|---|---|
| | | | | *cc.* | *sec.* | *mm.* | *min.* | *min.* |
| 7 | | 1 | Trachea. | 0.12 | 2 | 50 (100–150) | 6 | |
| | | 2 | " | 0.12 | 4 | 39 (88–127) | 12 | 9 |
| | Gray ♂ | 3 | Erector spinæ muscle (left). | 0.6 | 3 | 43 (99–142) | 20 | 13 |
| | 1,610 gm. | 4 | Trachea. | 0.12 | 8 | 21 (98–119) | 10 | 21 |
| Vagi intact. | | 5 | " | 0.12 | 5 | 25 (95–120) | 12 | 15 |
| | | 6 | Erector spinæ muscle (right). | 0.28 | No rise in 8 min. | | | 14 |
| | | 7 | Trachea. | 0.12 | 3 | 24 (92–116) | 21 | 8 |
| | | 8 | " | 0.24 | 10 | 10 (102–112) | 5 | 20 |
| 8 | | 1 | Trachea. | 0.21 | 6 | 64 (114–178) | 15 | |
| Vagi cut. | White ♀ 2,365 gm. | 2 | " | 0.21 | 10 | 30 (110–140) | 6 | 18 |
| | | 3 | " | 0.21 | 8 | 16 (116–132) | 9 | 8 |
| | | 4 | Erector spinæ muscle. | 0.42 | No rise in 4 min. | | | 15 |

Killed later by medullary puncture. Lungs collapsed well and showed only a moderate degree of pulmonary edema.

Pink fluid poured from trachea 13 min. after first dose. About 5 min. after last injection, blood pressure fell abruptly, convulsions, death. Lungs showed marked pulmonary edema.

TABLE III.

| No. of animal. | Color. Sex. Weight. | No. of injection. | Method and site of injection. | Amount of adrenalin per kilo. | Latent period of blood pressure rise. | Blood pressure rise. | Duration of blood pressure rise. | Interval between injections. |
|---|---|---|---|---|---|---|---|---|
| | | | | *cc.* | *sec.* | *mm.* | *min.* | *min.* |
| 4 | | 1 | Erector spinæ muscle (left). | 0.23 | 14 | 34 (106–140) | 4 | |
| | | 2 | Jugular vein. | 0.23 | At once. | 43 (107–150) | 10 | 9 |
| Vagi intact. | Gray 2,160 gm. | 3 | Trachea. | 0.23 | 8 | 20 (110–130) | 10 | 9 |
| | | 4 | Erector spinæ muscle (right). | 0.23 | No rise in 2 min. | | | 11 |
| | | 5 | Trachea. | 0.23 | 10 | 10 (100–110) | 3 | 2 |
| | | 6 | Erector spinæ muscles. | 0.23 | No rise. | | | |

After last injection foam and pink fluid poured from trachea (marked pulmonary edema).

A study of these typical protocols as given in Tables I, II, and III shows with clearness all the points we wish to emphasize, and they will now be considered in detail.

*Latent Period.*—The latent period elapsing between injection and the onset of the blood pressure rise varied between 2 and 38 seconds, but usually was less than 10 seconds; the general average of all first injections was 13 seconds. Repeated injections exerted no uniform effect on the length of the latent period. Thus in Experiment 7 the first dose of 0.12 cc. of adrenalin per kilo injected into the trachea surely reached a physiologically effective concentration in the blood within 2 seconds, for after that interval the blood pressure began to rise. The seventh injection of the same dose in the same place, 80 minutes later, exerted a blood pressure effect after a latent period of only 3 seconds, in spite of the fact that the animal had received intratracheally and intramuscularly in the interval between the two injections mentioned 1.24 cc. of adrenalin per kilo, divided into five doses. A still more striking example of the speed of absorption is

given in Experiment 8. In this experiment the vagi had been cut before administering the adrenalin, and the first dose of 0.21 cc. per kilo into the trachea caused a pulmonary edema of such an extent that foam and pinkish fluid poured from the tracheal cannula. Nevertheless, a repetition of the same dose in the same place a few minutes later exerted a good blood pressure effect after a latent period of only 10 seconds. In this instance the area available for absorption was undoubtedly reduced considerably by the pulmonary edema, yet the speed of absorption was still rapid. All the tabulated protocols show a similar rapid absorption after intratracheal injections.

The latent period or speed of absorption was not apparently affected by the amount of adrenalin injected; for example, in Experiment 3 the intrabronchial injection of 0.23 cc. per kilo by means of a catheter showed a latent period of 12 seconds before the blood pressure began to rise. In Experiment 7, on the other hand, a much smaller dose per kilo, 0.12 cc., injected into the trachea gave a blood pressure effect after a latent period of only 2 seconds.

*Quantitative Absorption.*—The quantitative absorption cannot be definitely established, yet the blood pressure rises obtained after the various injections furnish some indication of the amount of adrenalin absorbed. Examination of the absolute values of the blood pressure rises in mm. of mercury obtained after successive injections of the same dose of adrenalin in the same place, the lung passages for example, shows that the blood pressure effect in general decreases with the number of injections. Thus in Experiment 7 five doses of 0.12 cc. of adrenalin per kilo were injected into the trachea, the time intervals varying between 9 and 34 minutes if the interpolated intramuscular injections are included. The blood pressure rises after these five tracheal injections were 50, 39, 21, 25, and 24 mm. In Experiment 8 the successive decrease of effect is more pronounced. In this experiment 0.21 cc. of adrenalin per kilo into the trachea at intervals of 8 to 18 minutes gave the following rises of blood pressure: 64 ,30, and 16 mm. In this animal, however, the vagi had been sectioned previous to the first injection of adrenalin, and after the first dose a marked pulmonary edema developed during which fluid poured from the tracheal cannula. The presence of so much fluid decreased the absorptive area available, and in addition diluted the adrenalin with a colloidal

solution, rendering absorption still slower, yet in spite of this the speed of absorption was not appreciably delayed, the latent period being 10 and 8 seconds, but the amount absorbed, as judged by the pressure rises, showed practically 50 per cent decreases.

This decrease in effectiveness in causing a blood pressure rise shown by successive tracheal injections is in accord with the observations of Meltzer and Auer[4] that adrenalin diminishes absorption from the tissues. These authors demonstrated, among other facts, that the intravenous injection of adrenalin delays the absorption of strychnine or fluorescein from the subcutaneous tissue, and also that repeated intramuscular injections give a decreasing blood pressure effect, due to diminished absorption.

It will be noticed on examining the tables that all injections of adrenalin into the trachea gave some blood pressure effect; in other words, that some absorption took place even under unfavorable circumstances. A comparison was therefore made between the absorptive capacity of the lung and that of the erector spinæ muscles. The erector spinæ muscle was chosen because this thick mass of muscle is composed of fine fibers not separated into coarse fasciculi, like the glutei for example, and in addition is surrounded by a dense fascia which exerts pressure upon the injected substance, thus facilitating absorption. Injection into this muscle has been shown by Meltzer and Auer[4] to be practically equivalent to an intravenous injection.

The experimental test gave important results. It clearly appeared that the injection of several doses of adrenalin into the lung passages reduced the absorption from the erector spinæ muscles to such a degree that even double the intratracheal dose given intramuscularly did not enter the circulation in sufficient amount to affect the blood pressure; nevertheless, another injection of the original dose of adrenalin into the lungs promptly entered the circulation in sufficient concentration and amount to cause a blood pressure rise (see Experiments 3, 6, 7, and 4). This fact appears with especial clearness in Experiment 3. In this test the rabbit received three doses each of

[4] Meltzer, S. J., and Auer, J., *Tr. Assn. Am. Phys.*, 1904, xix, 207; *Jour. Exper. Med.*, 1905, vii, 59. Auer and Meltzer, *ibid.*, 1911, xiii, 328.

0.23 cc. of adrenalin per kilo into the lung, the time consumed being about 29 minutes. Each injection gave a rise of blood pressure (64, 45, and 25 mm.). Then the same dose was injected intramuscularly, but no blood pressure rise occurred in 4 minutes. The dose was then doubled and 0.46 cc. per kilo was injected into the other erector spinæ muscle, but again no blood pressure rise followed. 4 minutes later the original dose of 0.23 cc. was injected into the lung passages and after a latent period of 7 seconds the blood pressure began to rise and reached an absolute value of 24 mm. of mercury.

The failure to obtain a blood pressure effect from an intramuscular injection of adrenalin under the conditions mentioned is, however, not absolute. If the dose administered intramuscularly is increased sufficiently, enough adrenalin will be absorbed to cause a blood pressure rise. This is illustrated in Experiment 7. Two lung injections of 0.12 cc. of adrenalin each had been injected in about 22 minutes. Then five times the dose (0.6 cc. per kilo) was injected intramuscularly; after a latent period of 3 seconds the blood pressure rose 43 mm. That there was, nevertheless, a definitely diminished absorption from this large intramuscular injection is shown by the fact that the rise of blood pressure, 43 mm., is even less than that caused by the first lung injection of only one-fifth the dose, which latter raised the pressure 50 mm. Subsequent injections of adrenalin into the lungs all gave rises of blood pressure, but an interpolated intramuscular injection of double the pulmonary dose gave no blood pressure effect. The amount absorbed from the muscles had fallen below the level of a physiologically effective dose.

In some experiments the conditions were still further varied by preceding the lung injections of adrenalin by intramuscular and intravenous injections of the same substance. Experiment 4 is one of this type. The adrenalin dose was always 0.23 cc. per kilo. After an intramuscular and an intravenous injection, absorption of adrenalin from the lung was by no means prevented, the blood pressure rising 20 mm. after a latent period of 8 seconds. A subsequent intramuscular dose, however, produced no blood pressure rise within 2 minutes. Another lung injection even now caused a definite rise of pressure.

These experiments definitely show that, under the conditions

mentioned, absorption from the lungs occurs with doses of adrenalin which are ineffective when injected intramuscularly.

There was no definite relationship to be observed between the amounts of adrenalin injected into the lungs and the resultant blood pressure rise. The same dose per kilo often produced widely different rises in different animals, and smaller doses often caused greater elevations of pressure than larger ones. Table IV illustrates this and demonstrates in addition that the amount of adrenalin injected played no part.

TABLE IV.

| | | *gm.* | | | *mm.* |
|---|---|---|---|---|---|
| No. 5 | Vagi intact. | 1,650 | 0.3 cc. per kilo (0.5 cc.) | 4 sec. latent period. | 33 rise (107–140) |
| " 7 | " " | 1,610 | 0.12 cc. per kilo (0.2 cc.) | 2 " " " | 50 rise (100–150) |
| " 8 | " cut. | 1,980 | 0.25 cc. per kilo (0.5 cc.) | 18 " " " | 30 rise (114–144) |
| " 13 | " " | 1,790 | 0.25 cc. per kilo (0.4 cc.) | 18 " " " | 82 rise (116–198) |

*Blood Pressure.*—The duration of the blood pressure elevation was usually less than 10 minutes; if only the effects of the first tracheal injections are considered the average is 6 minutes. Subsequent injections, however, often showed a definitely longer duration of the elevation. An illustration of this effect will be found in Experiment 7.

The character of the rise varied somewhat; it was usually more or less abrupt, the maximum being reached within 30 seconds. In other instances the maximum elevation was reached in about 1 minute. The abruptness of the rise seemed to bear some relation to the number of preceding injections, the slope becoming less steep with succeeding doses. This, however, was by no means true of all experiments, for in some all intratracheal injections gave sharp rises of pressure. Vagus pulses were often observed, but their occurrence was not as frequent as when the adrenalin is administered intravenously or intramuscularly.

In a number of experiments abrupt and profound drops of blood pressure were noted. These occurred without any warning during the maximum pressure elevation, the pressure falling within a few

seconds to 20 mm. and even less. These drops lasted from 30 to 160 seconds, and were not necessarily fatal. In some instances a number of these profound drops occurred, recovery of the blood pressure taking place spontaneously after a series of convulsions. When these drops occurred, they were always associated with more or less pronounced signs of pulmonary edema. The significance of this phenomenon will be discussed in a later paper.

*Site of Absorption.*—In order to obtain some information regarding the site of absorption of the injected drug, a few experiments were made with intratracheal injections of India ink or suspensions of lampblack in an extremely dilute gum arabic solution. Doses of 0.3 cc. per kilo of body weight were injected slowly by syringe into the tracheal cannula. 10 seconds after the injection the medulla was destroyed by puncture, and the lungs and trachea were immediately excised and examined. The three experiments carried out gave concordant evidence: the posterior and diaphragmatic surfaces of the lower left lobe always showed a large number of discrete and confluent, irregular black spots varying from about 2 to 5 mm. in diameter. The surfaces of the other lobes showed only a few or no spots, and these were confined largely to the posterior surfaces near the hilus of the right lower and right middle lobes.

On sectioning the lungs through the trachea and bronchi, the larger part of the left lower lobe was found to be a black mass containing foam and some fluid. The right lower and right middle lobes near the hilus also contained an amount of pigment which was greater than would be expected from the surface indications. The upper lobes and the median lappet showed a few spots in the body of these divisions.

The sections of the lung containing the pigment were larger and fuller than those free from it; moreover, they contained more fluid. The distension was greater than the amount of fluid present explained, and apparently was at least partly caused by a mechanical plugging of the bronchioles and infundibular ducts by the pigment. The amount of fluid present in the tissues seemed greater than the amount injected (0.3 cc.), so that perhaps some degree of pulmonary edema also developed.

These experiments with the tracheal injection of pigment suspen-

sions thus indicate that a certain amount penetrates to the alveoli, chiefly of the left lower lobe, within less than 1 minute, and that absorption in all likelihood takes place there.

It might be thought that some absorption could take place from the mucosa of the trachea and the bronchi, and such absorption indeed does take place, at least as far as the tracheal mucosa is concerned. This absorption from the tracheal mucosa is, however, quite slow and the blood pressure rise obtained sets in very slowly. We tested the absorptive power of the tracheal mucosa in the following way. The tracheal cannula was inserted as low as possible in the neck; the upper section of the trachea was then ligated near the cannula, converting it into a sac. Injections of adrenalin were then made into this sac and the blood pressure effect was noted. In the two experiments made the first injection of adrenalin gave each time a slow and gradual rise of pressure, the latent period being respectively 150 and 52 seconds. In the first experiment the blood pressure rise equaled 59 mm. and lasted longer than 15 minutes. In the second experiment (Experiment 6) the first injection into the tracheal sac gave a rise of only 16 mm. of mercury after a latent period of 52 seconds, the rise persisting for more than 15 minutes. A subsequent repetition of the injection gave no blood pressure effect within 6 minutes. The doses of adrenalin injected were respectively 0.3 and 0.25 cc. per kilo.

These experiments show that while absorption of adrenalin does take place from the tracheal mucosa, and therefore probably also from the bronchi and bronchioles, these surfaces play only a subsidiary part as sites of absorption when adrenalin is injected into the trachea.

Section of the vagi in the neck before the intratracheal injection of adrenalin yielded interesting results. These nerves were cut in order to prevent the occurrence of those profound blood pressure drops mentioned previously, on the assumption that they were due to the well known initial effect of adrenalin upon the vagus center. However, these drops still occurred in some of the experiments after section of the vagi, and in addition, pulmonary edema and sudden death were much more frequent than in the series with vagi intact. One intratracheal injection of adrenalin often sufficed to bring on a strong pulmonary edema and even death within a few

minutes. Without entering here into a discussion of all the phenomena observed, it may be said, in general, that section of the vagi produced no noteworthy alteration in the absorptive power of the lung tissue as far as adrenalin is concerned. The variations observed in the vagotomized series fell well within the range of those seen in the normal series, though a percentage reckoning of all intratracheal injections given for the first time, shows that the vagotomized animal exhibited a shorter latent period (10 against 13 seconds), a higher blood pressure rise (56 against 46 mm.), and a longer duration of the pressure elevation (9.5 against 6 minutes). Not much stress, however, should be laid upon averages gained from only twenty-three experiments, especially in this work where pulmonary edema entered as a complicating factor in the vagotomy series.

A number of experiments were also carried out with a 0.1 per cent solution of adrenalin made from the commercial powder, a few drops of concentrated hydrochloric acid being added to the sterile saline to bring about solution. This solution, with or without the addition of chloretone, did not give as good results when injected intratracheally as the solution obtained in the open market; the absorption was slower and the resultant rise of blood pressure less marked.

A few experiments were also carried out with the sodium salt of fluorescein. Solutions of this substance, in 1 or 10 per cent strength, were injected intratracheally, the dose being 0.3 cc. per kilo of body weight. Samples of blood were then taken at regular intervals from the carotid artery, allowed to clot in small test-tubes, and the serum was examined for fluorescence. These experiments also indicated a rapid absorption though not as striking as with adrenalin; after 15 to 30 seconds the blood samples showed the first detectable green fluorescence. This fluorescence rapidly increased at first, then more slowly, reaching a maximum after a number of minutes.

## DISCUSSION AND SUMMARY.

In the preceding pages we have submitted evidence which shows that a simple intratracheal injection of a solution in a normally breathing rabbit penetrates within a few seconds to the alveoli, chiefly those of the left lower lobe; that absorption is rapid and well maintained;

and that the procedure may be repeated effectively a number of times even with a substance like adrenalin which decreases absorption. It was also shown that absorption of adrenalin from the lung could be obtained at a time when double the dose given intramuscularly exerted no blood pressure effect whatever, and that absorption could still take place after the development of pulmonary edema, when there was an undoubted dilution of the injected solution with a serum-containing liquid and when a diminution of the absorptive field had occurred.

The solution injected, after reaching the alveoli, is probably largely taken up by the capillaries of the pulmonary veins. This is indicated by the great rapidity with which an intratracheal injection of adrenalin may cause a rise of blood pressure. In numerous instances, for example, the pressure began to rise less than 5 seconds after the completion of an injection, equaling and even surpassing in rapidity of effect an intramuscular injection. Absorption by the lymphatics probably plays a secondary part, an assumption rendered all themore likely if we consider that lymph nodes are interpolated in the lymphatic pulmonary path, where the bed of the lymph stream becomes greatly widened and the current slowed.

Injection into the lungs, however, offers another advantage due to the vascular arrangement of the absorbing field which could be of value therapeutically. Absorption of liquids injected into the lung probably takes place largely through the capillaries of the pulmonary veins; to a slight extent possibly through the capillaries of the bronchial veins which empty partly into the pulmonary veins, partly into the azygos veins; and probably some absorption occurs also through the lymphatics. By far the larger proportion of the absorbed material will thus be rapidly delivered to the left auricle and then to the left ventricle. At each succeeding systole, as long as absorption continues, a fraction of the drug will be driven into the coronary arteries and be able to affect the musculature of the cardiac pump. This fact ought to render the procedure of intratracheal injection a valuable method when it becomes imperative to stimulate a suddenly failing heart as promptly as possible by drugs of the digitalis group.

Intratracheal injection is perhaps better under the conditions mentioned than the intravenous route, for the surface veins cannot

always be entered with promptness and certainty even under fairly normal conditions, and in cases of cardiac weakness the difficulties will be measurably increased, while an intratracheal injection can be carried out with ease. Moreover, it is legitimate to expect that some absorption will take place from the lung alveoli as long as the heart-lung circulation persists, no matter how feebly, and that thus some of the drug will reach the heart to act on this structure itself more promptly perhaps than when the drug is administered successfully through surface veins. As far as the intramuscular route is concerned, we have shown that the intratracheal injection of adrenalin gives prompt though diminished absorption at a time when double the dose intramuscularly exerts no blood pressure effect whatever.

The technical difficulties of giving an intratracheal injection in animals are slight. Tracheotomy as practised by us in the present series of experiments is not necessary, for the injection may be given into the intact trachea without exposure of the trachea. The hypodermic needle is inserted through the skin about 1 cm. below the larynx in a slanting caudad direction; the entrance of the needle into the trachea is readily felt. The injection should not be so rapid that the injected solution fills the entire tracheal lumen, but it should flow down the sides of the trachea. If the lumen is entirely filled, an expiration may drive some of the injected liquid into the larynx causing cough. In our experiments each injection of about 0.5 cc. consumed approximately 5 seconds.

In the human subject no data are available as far as our knowledge goes, but *a priori* it would seem that an intratracheal injection is almost as simple as in the lower animals. The free hypodermic needle could be inserted into the tracheal lumen immediately below the cricoid cartilage. The needle itself should preferably be connected with the syringe by a short length of rubber tubing to minimize the danger of breaking the needle by a sudden move of the patient. The amount of the solution should not be too small, so that at least a fraction of it may reach the alveoli as promptly as possible; 3 to 5 cc. probably would suffice.

Insertion of the needle in the locality mentioned would puncture the isthmus of the thyroid, but this is of no significance, especially when the procedure is employed in cases of cardiac failure where the

gravity of the condition would warrant incurring much heavier risks than a slight bleeding from the thyroidal isthmus.

In conclusion it may be said that the incorporation of drugs by intratracheal injection, while not as generally applicable as other methods, nevertheless has advantages which warrant its use also in human therapeutics.

# THE PRODUCTION OF AMYLOID DISEASE AND CHRONIC NEPHRITIS IN RABBITS BY REPEATED INTRAVENOUS INJECTIONS OF LIVING COLON BACILLI.

By C. H. BAILEY, M.D.

*From the Pathological Laboratory of the Medical School of Leland Stanford Junior University, San Francisco.*)

PLATES 110 to 115.

(Received for publication, March 14, 1916.)

The experiments here reported are a portion of a larger series in which several varieties of bacteria were used and which were undertaken in the hope of producing in rabbits by repeated intravenous injections of living bacteria, over long periods, kidney lesions which might at least approximate the chronic lesions seen in man, which are probably due to long continued septic conditions. The results in rabbits injected with *Bacillus coli communior* were so striking in the constant and pronounced production of amyloid in various organs, as well as in the resulting kidney lesions apart from the amyloid deposits, that a brief report on them and a review of previous work on the experimental production of amyloid seems appropriate.

Frisch (1) in 1877 was the first to announce the experimental production of an amyloid-like substance. He produced a keratitis in rabbits by injection into the cornea of fresh blood from a case of anthrax. He reports positive results in 4 out of 300 corneas injected. The resulting lesion gave characteristic reactions with iodine and with iodine and sulphuric acid, but the reaction with aniline dyes was lacking and the material was doubly refracting. It seems doubtful, therefore, whether the substance was amyloid.

Birch-Hirschfeld (2) observed amyloid in the spleen of a rabbit which died after 6 weeks of chronic subcutaneous suppuration produced by the injection of pus from a case of osteomyelitis of the tibia. Other similar experiments gave negative results.

Bouchard and Charrin (3) observed amyloid in two rabbits. One had been injected subcutaneously with *Bacillus pyocyaneus* and subsequently four times intravenously at intervals of several months. The animal lived about 1 year. Amyloid was found in the kidneys and also in the vessels of the heart and in the heart muscle. A second rabbit lived 34 days after injection with material from

a human tuberculous lesion and with a culture of tubercle bacilli. Amyloid was present in the kidneys; there was none in the spleen, liver, or heart.

Czerny (4) in his first paper reports the production of amyloid in the spleen and in a few vessels of the kidneys of two dogs in which he had produced subcutaneous suppuration for 10 and 13 weeks by the injection of turpentine. The material deposited gave the iodine-sulphuric and methyl violet reactions. Czerny's paper is concerned especially with the iodophilic granules of leukocytes from which he believes amyloid is formed. In a second paper (5) he reports the production of amyloid by the same method in three more dogs, in one after suppuration lasting 7½ months, and in two after 4 months.

Condorelli-Maugeri (6) in a brief communication simply states that he has produced amyloid disease in the liver and kidneys of rabbits by injection of *Bacterium termo*. As far as the writer can discover, no detailed account of his experiments has been published.

Krawkow (7, 8, 9, 10) was the first to study systematically the question in a large series of different animals. He produced chronic suppuration by the subcutaneous injection of broth cultures of *Staphylococcus aureus* into rabbits, dogs, hens, doves, and frogs. In eight of twelve rabbits he produced amyloid, mainly in the spleen, in certain instances also in the gastro-intestinal tract, liver, kidneys, and salivary glands. In dogs the results were negative after subcutaneous suppuration lasting 2 to 3 months. The results in pigeons were also negative. He found amyloid much easier of production and more extensive in hens than in other animals, three of four hens and each of four cocks giving positive results in 1½ to 2 months. In two frogs, of several dozen injected, the spleens showed traces of a substance giving a suggestive reaction, but Krawkow was not certain that it was amyloid.

Krawkow (9) also tried Czerny's method of injecting turpentine subcutaneously and also silver nitrate, both with negative results. He believes that the abscesses produced by Czerny were not aseptic. He also injected rabbits and dogs over long periods with filtrates of cultures of *Staphylococcus aureus* and also with non-filtered cultures killed at 100°C. with negative results. Amyloid was found, however, in one rabbit which in 2 months had received seventeen injections of 5 to 30 cc. each of filtrate of a broth culture of *Bacillus pyocyaneus* killed at 100°C. In this experiment amyloid was present in the spleen in the small arteries and to some extent about the capillaries. In the kidneys traces were found about the convoluted tubules, but none in the glomeruli. None was found in the liver. As pointed out by several later workers, Krawkow's results are surprising when one considers the length of time of his experiments. In two rabbits amyloid was found in the spleen 2 days after the first injection of 10 cc. of broth culture of *Staphylococcus aureus*. In a third amyloid was pronounced after 11 days, during which time two injections of 2 and 3 cc. had been given, while in other cases injected repeatedly over a period of months, it was entirely lacking.

Davidsohn (11) confirmed the observation of Krawkow of the possibility of producing amyloid by subcutaneous suppuration resulting from repeated in-

jections of *Staphylococcus aureus*. He used rabbits, mice, hens, guinea pigs, and cats. In guinea pigs and cats the results were negative; the best results were obtained with mice and rabbits. There is the same inconsistency in time and the amount of culture injected as in Krawkow's experiments. Amyloid was found in the spleen, stomach, intestines, kidneys, and rarely in the salivary glands, pancreas, and lymph nodes. In only one instance doubtful amyloid was found in the bone marrow. In the kidneys amyloid was present in patches in small arteries of the medulla and along the collecting tubules; there was no change in the glomeruli. He obtained negative results with streptococcus, *Bacillus coli*, and putrefactive bacteria. Davidsohn believes that Czerny's positive results with turpentine may have been due to bacterial contamination of the lesions.

Lubarsch (12) was at first unable to produce amyloid by Czerny's method. Later (13) he produced suppuration for 9 to 21 weeks in eight dogs by the injection of turpentine. In one case of 16 weeks' duration, he found amyloid in the spleen only. A second was doubtful, the others negative. Seven rabbits were injected repeatedly subcutaneously with living broth cultures of *Staphylococcus aureus*, the longest period being for 9½ weeks. Positive results were obtained in two cases, of 5½ and 7 weeks. Amyloid was found in the spleen only. Guinea pigs were injected with living *Staphylococcus aureus* and dead broth cultures of *Bacillus pyocyaneus*, pneumococcus, streptococcus, and Friedländer's bacillus with negative results. Lubarsch calls attention to the irregularity of results, especially as to time, of his own experiments and those of others, and concludes that no certain method of producing amyloid has been found.

In later papers Davidsohn (14, 15, 16) reviews his own work and that of others and reports the subcutaneous injection of mice with gonococcus with positive results in two cases. His experiments with bacterial toxins gave negative results. With dead bacteria, however, he obtained positive results in one mouse in which amyloid was found in the spleen, liver, kidneys, and intestines.

Nowak (17) attempted to prove and extend the work of Krawkow, and his results seem worthy of reviewing in some detail. He experimented on rabbits and hens and found it much easier to produce amyloid in the latter. He used staphylococcus, streptococcus, *Bacillus pyocyaneus*, *Bacillus coli communis*, and their sterile filtrates; bouillon inoculated with human stool and a filtrate of this; also tuberculin, fresh and sterile pus, croton oil, and turpentine. Of seven rabbits injected with living *Staphylococcus aureus*, two, of 10 and 102 days' duration, showed amyloid in the spleen only. All hens so injected showed amyloid. Two rabbits injected with living *Bacillus pyocyaneus* were negative; two hens were positive. Two rabbits injected with living *Bacillus coli* and also two hens showed no pus and no amyloid. Rabbits injected with putrefied bouillon gave negative results, while each of two hens were positive. Of the rabbits injected with sterile filtrates of cultures of various organisms, one rabbit only, which had been injected with a filtrate of a culture of *Bacillus pyocyaneus* for 60 days, showed a few nodular deposits in the spleen which gave the amyloid reaction with aniline colors. Of nine hens injected with sterile filtrates, three were positive; two of these had

been treated with filtrates of *Staphylococcus aureus* cultures, and one with a filtrate of cultures of *Bacillus coli*. Of two rabbits and two hens injected for long periods with large doses of tuberculin, one of each showed nodules in the spleen which gave a doubtful reaction. Nowak considers them negative. Of three rabbits injected with fresh pus, one, in 8 days, showed amyloid in the spleen and liver; of two hens similarly injected, one showed amyloid in the spleen and liver. Rabbits injected with sterile pus were negative; two hens were both positive. Croton oil gave negative results. Of two rabbits injected with turpentine, one showed amyloid in the spleen and liver in 201 days; each of five hens so injected showed amyloid in the spleen and liver, and two of these in the kidneys also.

Maximow (18) also obtained positive results in hens and rabbits by repeated subcutaneous injections of living *Staphylococcus aureus*. His earliest positive result was in a rabbit after 21 days.

Schepilewsky (19) produced extensive subcutaneous necrosis and suppuration by the injection of various ferments. Out of a considerable number of experiments he reports positive results in two rabbits injected with lab-ferment, in one injected with pancreatin, and in one with papayotin. The author makes the criticism of the positive results of Lubarsch and Nowak with turpentine, and of those of Krawkow, who obtained a positive result in one rabbit with *Bacillus pyocyaneus* toxin, and of Davidsohn, who obtained a positive result in one mouse injected with dead bacteria, that bacterial contamination of the suppurating lesions produced was not excluded. Davidsohn (14, 16) on the other hand, believing that amyloid cannot be produced without the action of living bacteria or their products, makes a similar criticism of Schepilewsky's results with ferments, and apparently with equal justice. The latter admits that the abscesses in one of the four positive cases were not sterile. The other three he believes were sterile, but each of these animals died of an epidemic disease of a nature not stated, and one of the three had a purulent bronchitis.

Dantschakow (20) studied the salivary glands of rabbits injected subcutaneously with living *Staphylococcus aureus*. She believes that amyloid first appears here. She found it in some cases in the 2nd week, in others not until the 6th or 7th.

Tarchetti (21) reports entirely negative results. He injected a dog subcutaneously with *Staphylococcus aureus*. When the animal had become immune and the injections no longer produced abscesses, he used turpentine. The duration of the experiment was 4 months. Three rabbits injected subcutaneously with turpentine and eight with living *Staphylococcus aureus* were negative. He concludes that repeated injections of turpentine or broth cultures of *Staphylococcus aureus* produced no amyloid in the spleen of dogs, rabbits, or guinea pigs.

Ravenna (22) concludes from many experiments that the question of experimental production of amyloidosis still cannot be considered as solved, as a means is lacking which will produce it with certainty.

Of special interest in connection with the experiments here reported are the

observations of amyloid in the liver of horses repeatedly injected with bacteria or their toxins for the production of immune serum.

Zenoni (23) first reported the presence of amyloid degeneration in diphtheria antitoxin horses, especially in the liver. The lesion produced a very friable organ and death was apt to occur from spontaneous rupture and hemorrhage.

Pease and Pearce (24) report liver necrosis and venous thrombosis in twelve horses which had been repeatedly injected subcutaneously, seven with diphtheria toxin, two with tetanus toxin, one with dysentery bacilli, and two with streptococci. A horse is also added to the series which had been injected with diphtheria toxin and killed on account of its crippled condition. Its organs were normal. Two of these horses, both injected with diphtheria toxin, showed amyloid in the liver and spleen, especially in the latter. It was not present in the kidneys, heart, or lymph nodes. They note that these two animals had developed an unusual number of abscesses at the sites of injection, apparently attributing the amyloid formation to this fact.

Lewis (25) reports that amyloid degeneration of the liver and less frequently of the spleen is produced in a majority of horses by the routine injection of diphtheria toxin and repeated bleeding, extending over a period of 3 years. The horses are apt to die from rupture of the liver and intraperitoneal hemorrhage. He rules out abscesses as an essential condition and also the repeated bleeding, and concludes that the crude toxin is the factor of prime importance in the production of amyloid.

Schoukewitch (26) reports that repeated injections of large doses of *Bacillus pestis* produce amyloid degeneration of different organs in horses and that pus formation is not essential.

Markus (27) observed amyloid in the liver of horses injected with dysentery bacilli.

## EXPERIMENTAL PART.

In the following experiments rabbits were injected in the ear vein with beef extract broth cultures of colon bacillus, transferred from stock cultures on beef extract agar. The rabbits were injected, generally every 2 to 4 days, with 1 cc. of a 24 to 48 hour culture, though older cultures were occasionally used. Colon S 232 and Colon S 244 were strains of *Bacillus coli communior* obtained in pure culture from surgically removed pyonephrotic kidneys. Colon B was also *Bacillus coli communior* isolated from a normal stool.

Frozen sections of tissues fixed on Orth's fluid were stained with hematoxylin and Sudan III and also by Van Gieson's method. Sections of paraffin-imbedded tissues were also stained by Van Gieson's method. Tissues were stained for amyloid with iodine, iodine and sulphuric acid, methyl violet, and gentian violet. As has

been previously mentioned, the finding of amyloid in these rabbits was unexpected. The hyaline bodies in the kidney glomeruli and other glomerular and parenchymatous changes had been noted, but that the bodies in the glomeruli were of amyloid nature was not recognized until a majority of the rabbits had come to autopsy and pieces of their organs had been fixed in Orth's fluid only. Consequently it was generally possible to try the various reactions only under the relatively unfavorable conditions of previous fixation in Orth's fluid. In one rabbit, No. 15, the reactions were tried on fresh and alcohol-fixed tissues; in Rabbits 1, 3, and 7, on alcohol-fixed tissues; in all others, on tissues fixed in Orth's fluid only. The reactions were tried under these conditions on the spleen, liver, and kidneys of all rabbits. Pieces of other organs, however, which are of interest in connection with amyloidosis had not been preserved in most cases, and it is therefore impossible to report the presence or absence of amyloid degeneration in other situations except in a few instances. Table I shows the strain of bacteria used, the duration of the experiment in days, the number of injections, and the presence or absence of amyloid in the spleen, liver, and kidneys. Positive findings in other organs will be mentioned later.

TABLE I.

| Rabbit No. | Bacteria. | Duration of experiment. | No. of injections. | Amyloid. |
|---|---|---|---|---|
| | | *days* | | |
| 1 | Colon S 232 | 21 | 7 | None. |
| 2 | " S 232 | 26 | 9 | " |
| 3 | " S 232 | 30 | 11 | " |
| 4 | " S 232 | 30 | 10 | " |
| 5 | " S 244 | 59 | 8 | " |
| 6 | " S 232 | 61 | 15 | " |
| 7 | " S 232 | 63 | 19 | " |
| 8 | " S 232 | 88 | 23 | Spleen, liver, kidney. |
| 9 | " S 232 | 98 | 25 | " |
| 10 | " S 232 | 102 | 25 | " |
| 11 | " S 232 | 113 | 29 | " liver, kidney. |
| 12 | " B | 115 | 27 | " kidney. |
| 13 | " S 232 | 116 | 36 | " " |
| 14 | " S 232 | 142 | 30 | " " |
| 15 | " S 232 | 145 | 47 | " liver, kidney. |

Complete autopsies were performed on all the animals, which, however, will not be reported in detail, as the gross pathological findings were few. Suppurative lesions were lacking with the following exceptions: Rabbit 3 had an abscess about 1.5 cm. in diameter in the upper anterior mediastinum, and Rabbit 14 had a few small nodular areas of consolidation in the lungs, each about 3 mm. in diameter with central softening. All the rabbits lost much in weight, emaciation being extreme in the older cases. The livers were of about normal size and no change in consistency was noted except in No. 15, in which the organ was definitely firmer than normal but more friable. All the spleens were somewhat enlarged; in No. 14 the organ was about four times normal size, in No. 9 about three times, in Nos. 1 and 11 about twice; in the others the enlargement was slight. Beyond the enlargement there was no marked change in appearance or consistency. The gross appearance of the kidneys was not striking. In all the animals they were definitely opaque, in many very pale, and in some apparently swollen. Small, slightly depressed, purple or gray surface scars were present in many instances, in some being moderate in number, in most very few. None were present in Nos. 1, 2, 3, 4, 5, and 7. They had the same appearance as the spontaneous scars in rabbits, and it is difficult to decide whether they are to be ascribed to the treatment or to a preexisting spontaneous nephritis. The fact that they were absent in the experiments of shorter duration would favor the former view. The number and severity of the scars when present, however, definitely bore no relationship to the length of time the rabbit had been injected, the scars in Nos. 6, 9, and 10 being more numerous than in the older rabbits, in which they were very few and slight.

Microscopical examination of the spleens of the earlier rabbits, Nos. 1 to 7 inclusive, showed a large amount of hematogenous pigment collected in large phagocytic cells in the sinuses of the pulp. In some there was a slight diffuse fibrosis. Otherwise the spleens were normal. In Rabbits 8 to 15 the hematogenous pigment was also present and in all there was a fibrosis. All showed about the Malpighian bodies a homogeneous hyaline substance which in the more marked cases, Nos. 8, 11, 12, and 13, encroached on these structures (Fig. 4) and almost entirely replaced them, only a small

collection of lymphoid cells remaining grouped about the central artery. A moderate amount of nuclear detritus was present in this material, and in No. 8 numerous fairly large hemorrhages were present in the midst of the hyaline material, which had largely replaced the Malpighian bodies. Similar hyaline material was also seen in some animals in the reticulum of the pulp. In Rabbits 14 and 15 the fibrosis and hyaline formation, though more marked about the Malpighian bodies, was also present through the reticulum of the pulp (Fig. 5). Stained with methyl violet or gentian violet, this hyaline material, both about the Malpighian bodies and in the pulp, gave a typical red amyloid reaction in all cases (Fig. 1). It appeared in the form of small irregular masses which usually were quite homogeneous, but sometimes suggested a finely granular structure. When present in the reticulum of the pulp it had a definite fibrillar appearance. With iodine no reaction was obtained even in the fresh and alcohol-fixed tissues of No. 15. With iodine followed by sulphuric acid the larger collections about the Malpighian bodies gave a pale but definite greenish color. These reactions will be considered later. Large phagocytic cells were quite numerous in the spleen of these rabbits, and in No. 15 fairly numerous giant cells were present, having many nuclei and often including masses of the amyloid (Fig. 1). These cells have been previously noted by Krawkow (7).

All the livers showed a definite but usually moderate increase of periportal connective tissue and also an infiltration with round cells in these areas. It is difficult to decide whether this is a spontaneous cirrhosis or is due to the treatment. There were also present in all the animals a varying number of small focal necroses situated indiscriminately in the lobules. In the earlier ones these were infiltrated with round cells; in the later ones they were often replaced by connective tissue. There was considerable granular degeneration of the liver cells in these rabbits and also pyknosis of nuclei. In No. 13 numerous large central necroses were present with much fibrin and some hyaline degeneration but little cellular infiltration. The livers of Rabbits 8, 11, and 15 showed between the walls of the capillaries and the trabeculæ of liver cells a layer of hyaline material with atrophy and granular degeneration of the intervening liver cells. This was present in all lobules, but in No. 8 was seen only in the peripheral and middle

zones of the lobules, while in the other two it was fairly uniform throughout. With iodine and iodine followed by sulphuric acid, no reaction was obtained. With methyl violet and gentian violet this material gave a typical amyloid reaction. With these stains the substance appeared usually in a definite granular form (Fig. 2), but sometimes a fine fibrillar structure was apparent. The necroses with hyaline degeneration described in No. 13 gave no reaction. No amyloid was found in the walls of the larger vessels.

The kidneys of these rabbits deserve special attention, since they showed not only amyloid deposits but also other lesions definitely due to the bacterial injections. Degenerative changes were present in the tubular epithelium in all cases. Frozen sections stained with Sudan III showed a moderate fatty degeneration affecting principally the convoluted tubules. The fatty degeneration was, however, more marked in the earlier than the later cases. The was a definite granular degeneration, also most marked in the convoluted tubules, and some necrosis of the epithelium with pyknosis of nuclei, lack of nuclear staining, and desquamation. The large granules seen in the epithelium of the proximal convoluted tubules in chronic parenchymatous nephritis in man were not observed. Casts were present in all kidneys except those of Nos. 1 and 2, and in Nos. 13, 14, and 15 they were numerous. No glomerular changes, other than hyperemia, were noted in Rabbits 1 to 7, while all the others showed evidences of a subacute and chronic glomerulitis. The lesions consisted of fibrous and hyaline thickening of the vascular loops and occasionally of the glomerular capsules, protoplasmic, cellular, and fibrous adhesions between the tufts and capsules, areas of necrosis in the tufts, and hyaline bodies containing a few pyknotic nuclei and some nuclear débris. These lesions varied in number and degree in the different rabbits. Adhesions were present in Nos. 8 to 15 inclusive, being, however, few in Nos. 9 and 12 but numerous in the others, especially Nos. 13 and 14 (Fig. 6). Almost every glomerulus in No. 13 showed adhesions. Some of these adhesions were fine protoplasmic unions between tuft and capsule (Fig. 7), others large and cellular or fibrous (Figs. 7 and 8). Fibrous and hyaline thickening of the vascular loops was present in Nos. 8, 11, 12, 13, 14, and 15, with necroses and large amyloid bodies in some instances (Figs. 7, 8, and 9). The fre-

quent occurrence of localized interstitial scars in the kidneys of rabbits unfortunately renders the interpretation of interstitial lesions in these animals difficult. As has been already mentioned, most of the rabbits which withstood the injections longest showed a few small scars in the gross. There were usually only two or three such scars on a kidney. These scars have been disregarded as perhaps due to a preexisting spontaneous nephritis. In most of the kidneys, however, there were present small collections of round cells, and in Nos. 13, 14, and 15 there was a slight, rather diffuse, cellular thickening of the interstitial tissue. It is believed that these lesions were produced experimentally.

Six of the kidneys, as shown in the table, gave a typical amyloid reaction with methyl violet. The amyloid was present in the glomeruli as small and large nodular collections (Fig. 3) and also in the medulla where in some instances it was situated only about the small capillaries in small localized areas; in others it involved the entire interstitial tissue, in similar small nodular areas, here surrounding both vessels and tubules. No amyloid was noted in the larger vessels. Here as in other organs it was difficult to convince one's self definitely of the morphology of the amyloid. In certain instances the large deposits in the glomeruli seemed to have a definite fibrillar structure with a frayed out appearance at the edges, especially noticeable in the alcohol-fixed tissue of No. 15. In some of the other cases these bodies appeared quite homogeneous (Fig. 3). With iodine no reaction was obtained even with frozen sections of the fresh tissue of No. 15. With iodine and sulphuric acid the larger bodies in the glomeruli, like the deposits in the spleen, gave a pale but definite greenish reaction.

By reference to the table one notes that amyloid was present in the spleen of all rabbits, eight in number, which were injected for a period of 88 days or longer. These eight rabbits showed glomerular and other lesions in the kidneys, and in six amyloid was present. The livers of three contained amyloid. Organs other than the spleen, liver, and kidneys were examined for amyloid in only a few instances. The stomach, small intestine, large intestine, heart, adrenal, and submaxillary gland in No. 3 gave no reaction. The small intestine, large intestine, heart, and submaxillary gland in No. 7 gave no

reaction. The stomach and small intestine of No. 15 showed typical deposits in small scattered areas in the mucosa only; small irregular homogeneous bodies in the bone marrow gave a doubtful reaction with methyl violet; the heart and adrenals showed none.

## DISCUSSION.

It seems justifiable to regard the deposits described in these animals as amyloid in spite of the absence of the iodine reaction. It was possible to try the reaction on fresh or alcohol-fixed tissues in only one, No. 15, of the eight rabbits which showed deposits. There is a possibility that the reaction might have been obtained in some of the other animals under these conditions. It is the consensus of opinion, however, of those who have studied the question carefully, that the reaction with aniline colors is the more typical and that the iodine and iodine-sulphuric reactions are sometimes lacking. Davidsohn (11) and others believe that these reactions represent different stages in the development of amyloid, the reaction with aniline colors appearing earliest and being present throughout, the iodine reaction appearing next, and that with iodine and sulphuric acid representing the latest stage. Krawkow (10) and Maximow (18) report results similar to the above with the amyloid experimentally produced by them in animals. Krawkow (10) found the reaction with methyl violet constant, but obtained the iodine reaction in only a few instances, and then only on fresh or alcohol-fixed tissues, and in the latter it disappeared after 24 hours' fixation. Similar absence of the iodine reaction in amyloid deposits in man has been reported by Hansemann (28), Krawkow (10), and others. Krawkow (10) believes that these reactions do not represent a difference in chemical composition of the substance, but are due to a difference in physical conformation of the older deposits. The presence of the iodine-sulphuric reaction in the absence of the simple iodine reaction appears from the record of previous experiments to be exceptional. The possibility suggested itself that the green color obtained in these cases might be due to the presence of cholesterol in these degenerated areas. The large deposits in the kidneys and spleen, however, give no suggestion of a fat reaction with Sudan III, as one would expect were

the cholesterol present in any considerable amount in lipoid form; and the fact that the reaction was also obtained on specimens imbedded in paraffin would seem to rule out cholesterol as the responsible factor.

Scrapings of the fresh liver, spleen, and kidneys of Rabbits 3, 6, 8, 11, 13, and 14 were examined with the polarizing microscope for anisotropic fat. This examination is of interest on account of the reports of the presence of doubly refracting lipoids in human nephritis, particularly in the chronic parenchymatous type which these kidneys so closely resemble. The results were negative except in the liver of No. 6 and the kidneys of No. 8, in both of which a moderate number of small anisotropic droplets were present.

One notes that in the spleen there is an association of amyloid formation with evidences of blood destruction, cell necrosis, and fibrosis. Also in the glomeruli of the kidneys one sees necrosis and fibrosis associated with the deposition of amyloid. In the liver this association is not so evident. It is not justifiable to draw definite conclusions as to a possible relationship between amyloid formation and any one of these other phenomena. While the cell destruction may in part be the result of the deposition of amyloid, or the two may be mutually independent results of the toxic action, we believe that the possibility of a genetic relationship between cell necrosis and the formation of amyloid should not be too readily dismissed. Mallory's views are worthy of attention in this regard. He states (29) that amyloid in man when first deposited is finely fibrillar but soon becomes homogeneous and that it is not a product of degeneration of cell or fibril, nor is it something filtered out of the blood-like serum, but it is an abnormal product of the fibroblast. His argument that it is more reasonable to regard amyloid as the product of the fibroblast, which normally produces fibrils, than of the endothelial cell which does not, is forcible. He also calls attention to the fact that chondroitin sulphuric acid is a constituent of some of the normal products of these cells; namely, elastic fibers and chondromucin. As between its formation by endothelial cells and fibroblasts, these facts are certainly in favor of the latter. As previously noted, its fibrillar appearance in the spleen and kidneys of these rabbits is sometimes quite evident. This fibrillar appearance, however, does not seem to the writer good evidence that it is produced

as such by either fibroblasts or endothelial cells. The variation in the morphological appearance of the amyloid in different situations in these rabbits has already been noted. One is also impressed in reviewing the literature with the variations in appearance as described by different authors, some believing it to be fibrillar or homogeneous, others granular, and others crystalline. Of the various theories advanced as to the nature of this substance, the idea that it is a homogeneous, semisolid, infiltrating substance would seem to explain these variations in morphological appearance better than any other, variations being produced, as they are particularly apt to be in colloidal materials, by postmortem changes or the processes of fixation, and being less easily produced in older deposits, perhaps because of greater inspissation. If such a substance were produced or deposited in narrow tissue spaces, it might well appear in fixed specimens as thin sheets or fibrils. With atrophy of intervening collagen fibrils, which Mallory describes as occurring following the appearance of the amyloid fibrils, the infiltrating material might well fuse to form the homogeneous masses of the later stages.

Living bacteria only were tried in the experiments here reported. That the injection of dead colon bacilli alone might result in amyloid deposits is suggested by the results of Davidsohn (16) on a mouse injected with dead bacteria, and that toxins or decomposition products of the culture media might produce it is suggested by the results of Krawkow (7) and Nowak (17), each on a rabbit injected with filtrate of a culture of *Bacillus pyocyaneus* and by those on horses immunized with diphtheria toxin. These are questions not answered by these experiments. That it could be due to the normal constituents of beef extract broth would seem improbable. That this is not the case is evidenced by Rabbit 6 which had received twenty-five intravenous injections of sterile broth in a period of 101 days before the bacterial injections were begun. The period of injection of sterile broth was thus not as long as that of some of the rabbits which showed amyloid, but longer than in two of the rabbits, and about the same as that of a third which showed definite amyloidosis.

The question as to whether the ability to produce amyloidosis is a common property of all pathogenic bacteria or is confined to certain species or classes, is of considerable practical interest and also

one which has not been answered. From a study of previous work it seems evident, in spite of conflicting reports, that subcutaneous suppuration produced by living *Staphylococcus aureus* in hens and rabbits may, especially in the former, ultimately result in the formation of amyloid deposits. The positive results reported with other organisms are so few and the variety of animals on which these results were obtained is so great, that it is difficult to draw conclusions in this regard. The criticism made of the experiments in which subcutaneous suppuration has been produced by turpentine, ferments, and bacterial toxins,—that bacterial contamination was not excluded,—seems justified. The difficulty of keeping such subcutaneous lesions uncontaminated, especially with staphylococcus which we know will produce amyloid, over periods of weeks or months is obvious. Attention has already been called, in reviewing the literature, to the contradictory reports as regards the possibility of producing amyloidosis by subcutaneous suppuration and also to the inconsistency, as far as time is concerned, in the experiments of the various investigators who have reported positive results. In contrast with previous work the constant results here reported suggest the importance of bacteriemia in the production of amyloid and afford a possible explanation of the varying results in previous experiments, that in some cases bacteriemia resulted while in others the infection remained localized. Certain results reported, particularly those on horses, indicate that a toxemia is sufficient, without actual bacteriemia.

The author has previously stated that the experiments here reported with colon bacilli are a part of a larger series in which various bacteria were used. The only rabbit injected with other varieties of bacteria which lived 88 days or longer (that is, longer than the shortest period in which amyloid was produced with colon bacilli) was one injected with *Bacillus typhosus*. This animal received forty injections of 1 cc. each of broth culture in a period of 125 days. Another rabbit received twenty-three injections of 1 to 2 cc. each in a period of 84 days. Neither of these rabbits showed amyloid deposits. One would not be justified from these two instances in drawing definite conclusions, yet the results would seem to indicate that at least there is a marked difference in the facility with which amyloid is produced by different organisms. The principal other bacteria used were

various strains of streptococci. Amyloid was not produced, but inasmuch as the duration of the longest experiments was 87 days (ten injections) and 78 days (twenty-two injections) comparative conclusions in these instances also are not warranted. If experimental investigation should prove that amyloid is produced more easily by some varieties of organisms or their products than by others, a careful investigation of the numbers and varieties of bacteria present in pulmonary cavities in chronic tuberculosis would also be of interest as possibly affording an explanation of the recognized fact that amyloidosis is extreme in some cases of chronic tuberculosis while entirely lacking in others of equal duration and extent of lesions. The explanation which has been offered of individual and racial susceptibility seems inadequate to explain the marked differences seen in this regard. At least the explanation is one to which we should not resort until the above possibility has been excluded.

## SUMMARY.

The repeated intravenous injection of rabbits with living *Bacillus coli communior* over long periods has resulted in the formation of amyloid deposits in the spleen, liver, and kidneys. Suppurative lesions were not present in most cases and therefore not a factor in its production. The results have been constant in that amyloid was found in all rabbits, eight in number, which were injected over a period of 88 days or more. Eight rabbits showed amyloid in the spleen, six of these in the kidneys also, and three in the liver.

The kidneys of these eight rabbits also showed as a result of the injections a subacute and chronic glomerulitis, parenchymatous degeneration, some interstitial infiltration with round cells, and a slight cellular proliferation of connective tissue, thus resembling the chronic parenchymatous nephritis of man which is so commonly associated with amyloid disease.

In conclusion I wish to express my appreciation of the advice and assistance of Dr. Ophüls throughout this work.

BIBLIOGRAPHY.

1. Frisch, A., Ueber eigenthümliche Produkte mykotischer Keratitis mit der Reaction des Amyloids, *Sitzungsb. d. k. Akad. d. Wissensch. Math. naturw. Cl., 3te Abt.*, 1877, lxxvi, 109.
2. Birch-Hirschfeld, F. V., Lehrbuch der pathologischen Anatomie, Leipzig, 1882, i, 45.
3. Bouchard and Charrin, Dégénérescence amyloïde expérimentale, *Compt. rend. Soc. de biol.*, 1888, v, 688.
4. Czerny, A., Zur Kenntniss der glycogenen und amyloïden Entartung, *Arch. f. exper. Path. u. Pharm.*, 1893, xxxi, 190.
5. Czerny, A., Ueber die an Thieren experimentell hervorgerufene Amyloidentartung, *Centralbl. f. allg. Path. u. path. Anat.*, 1896, vii, 282.
6. Condorelli-Maugeri, A., Ueber die Aetiogenese der Amyloiddegeneration, *Centralbl. f. allg. Path. u. path. Anat.*, 1894, v, 417.
7. Krawkow, N.-P., Ueber bei Thieren experimentell hervorgerufenes amyloid, *Centralbl. f. allg. Path. u. path. Anat.*, 1895, vi, 337.
8. Krawkow, N.-P., De la dégénérescence amyloïde et des altérations cirrhotiques provoqués expérimentalement chez les animaux, *Arch. de méd. expér.*, 1896, viii, 106.
9. Krawkow, N.-P., De la dégénérescence amyloïde et des altérations cirrhotiques provoqués expérimentalement chez les animaux, *Arch. de méd. expér.*, 1896, viii, 244.
10. Krawkow, N.-P., Beiträge zur Chemie der Amyloidentartung, *Arch. f. exper. Path. u. Pharm.*, 1898, xl, 195.
11. Davidsohn, C., Ueber experimentelle Erzeugung von Amyloid, *Virchows Arch. f. path. Anat.*, 1897, cl, 16.
12. Lubarsch, O., Beiträge zur Histologie der von Nebennierenkeimen ausgehenden Nierengeschwülste, *Virchows Arch. f. path. Anat.*, 1894, cxxxv, 149.
13. Lubarsch, O., Zur Frage der experimentellen Erzeugung von Amyloid, *Virchows Arch. f. path. Anat.*, 1897, cl, 471.
14. Davidsohn, C., Die Rolle der Milz bei der Amyloiderkrankung, *Verhandl. d. deutsch. path. Gesellsch.*, 1904, vii, 39.
15. Davidsohn, C., Allgemeine pathologische Morphologie und Physiologie. I. Arbeiten über Amyloid und Hyalin, 1899–1907, *Ergebn. d. allg. Path. u. path. Anat.*, 1908, xii, 424.
16. Davidsohn, C., Untersuchungen über die Aetiologie des Amyloids, *Virchows Arch. f. path. Anat.*, 1908, cxcii, 226.
17. Nowak, J., Experimentelle Untersuchungen über die Aetiologie der Amyloidosis, *Virchows Arch. f. path. Anat.*, 1898, clii, 162.
18. Maximow, A., Ueber die experimentell hervorgerufene Amyloid-Entartung der Leber, *Virchows Arch. f. path. Anat.*, 1898, cliii, 353.
19. Schepilewsky, E., Experimentelle Beiträge zur Frage der amyloiden Degeneration, *Centralbl. f. Bakteriol., 1te Abt.*, 1899, xxv, 849.

20. Dantschakow, W., Über die Entwicklung und Resorption experimentell erzeugter Amyloidsubstanz in den Speicheldrüsen von Kaninchen, *Virchows Arch. f. path. Anat.*, 1906, clxxxvii, 1.
21. Tarchetti, C., Untersuchungen über experimentelle amyloide Entartung,
22. Ravenna, E., Ueber die experimentell erzeugte Amyloiddegeneration, *Centrabl. f. allg. Path. u. path. Anat.*, 1903, xiv, 677.
23. Zenoni, C., Toxische Amyloidosis bei der antidiphtherischen Immunisation des Pferdes, *Centralbl. f. allg. Path. u. path. Anat.*, 1902, xiii, 334.
24. Pease, H. D., and Pearce, R. M., Liver Necrosis and Venous Thrombosis in Horses Actively Immunized with Diphtheria and Tetanus Toxins and with Streptococci and Their Products, *Jour. Infect. Dis.*, 1906, iii, 619.
25. Lewis, P. A., Hemorrhagic Hepatitis in Antitoxin Horses, *Jour. Med. Research*, 1906, xv, 449.
26. Schoukewitch, J., De la dégénérescence amyloïde chez les chevaux soumis aux injections répétées de bacilles pesteux, *Arch. d. Sc. biol.*, 1907, xii. 190.
27. Markus, H., Over amyloide Degeneratie van de Lever bij het Paard, *Tijdschr. v. veeartsenijk. Maanbl.*, 1904–05, xxxii, 99.
28. Hansemann, Amyloider Degeneration nach Syphilis, *Deutsch. med. Wchnschr.*, 1893, xix, 582.
29. Mallory, F. B., The Principles of Pathologic Histology, Philadelphia, 1914, 102–107.

## EXPLANATION OF PLATES.

### PLATE 110.

FIG. 1. Rabbit 15. Spleen. A portion of a Malpighian body is shown which was surrounded and partially replaced by amyloid. A giant cell is seen, at the upper end of the picture, with amyloid inclusions. Camera lucida water color drawing by Dr. F. E. Blaisdell. Zeiss obj. E., oc. 1. Stained with methyl violet.

FIG. 2. Rabbit 15. Liver. The picture shows a considerable deposit of amyloid between the capillary walls and liver cells with granular degeneration and atrophy of the latter. The amyloid has a finely granular appearance. Camera lucida water color drawing by Dr. F. E. Blaisdell. Zeiss obj. E., oc. 1. Stained with gentian violet.

### PLATE 111.

FIG. 3. Rabbit 14. Kidney. The upper glomerulus was of homogeneous appearance with loss of normal structural detail and contained many pyknotic nuclei and a large homogeneous appearing amyloid body. The lower glomerulus showed a general thickening of the vascular loops with two small amyloid nodules. There were adhesions in both cases between tuft and capsule. Camera lucida water color drawing by Dr. F. E. Blaisdell. Zeiss obj. E., oc. 1. Stained with methyl violet.

PLATE 112.

FIG. 4. Rabbit 12. Spleen. A Malpighian body is shown surrounded and encroached upon by amyloid formation; also thickening of and deposition of amyloid in the reticulum of the pulp. Microphotograph. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

FIG. 5. Rabbit 14. Spleen. The photograph shows a fibrosis affecting the Malpighian body and also the reticulum of the pulp. There is some amyloid about the Malpighian body, but it is relatively more pronounced in this case in the reticulum of the pulp. , Microphotograph. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

PLATE 113.

FIG. 6. Rabbit 13. Kidney. The photograph shows eight glomeruli in each of which there are adhesions between tuft and capsule. Two glomeruli, the one at the top of the picture in the middle and also that at the bottom, show large amyloid bodies. Microphotograph. Bausch and Lomb obj. $\frac{2}{3}$, oc. 1.

PLATE 114.

FIG. 7. Rabbit 13. Kidney. A higher magnification of the glomerulus seen at the top of Fig. 6. Note the fine protoplasmic and large cellular adhesions, the hyaline body, and the swelling and slight proliferation of the lining cells of the capsular space. In an adjacent section of this same glomerulus the hyaline body gave a typical amyloid reaction with methyl violet. Microphotograph. Bausch and Lomb obj. $\frac{1}{6}$, oc. 1.

PLATE 115.

FIG. 8. Rabbit 13. Kidney. A glomerulus from the same rabbit but not in the field of Fig. 6. Note the extensive adhesions and the large hyaline body containing some nuclear débris. In an adjacent section the hyaline body in this glomerulus gave a typical amyloid reaction with methyl violet. Microphotograph. Bausch and Lomb obj. $\frac{1}{6}$, oc. 1.

FIG. 9. Rabbit 15. Kidney. Note the large body in the glomerulus with a few pyknotic nuclei and some nuclear débris. In an adjacent section of this same glomerulus the entire body gave a typical amyloid reaction, the material appearing in a distinctly fibrillar and finely granular form. Microphotograph. Bausch and Lomb obj. $\frac{1}{6}$, oc. 1.

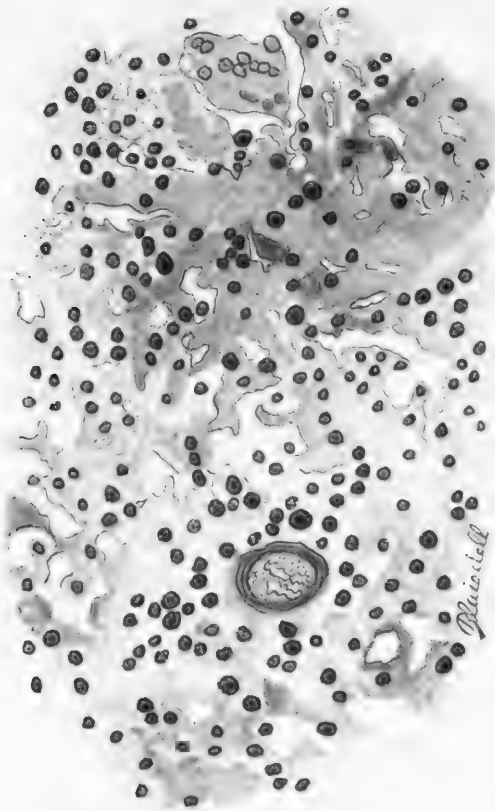


FIG. 1.

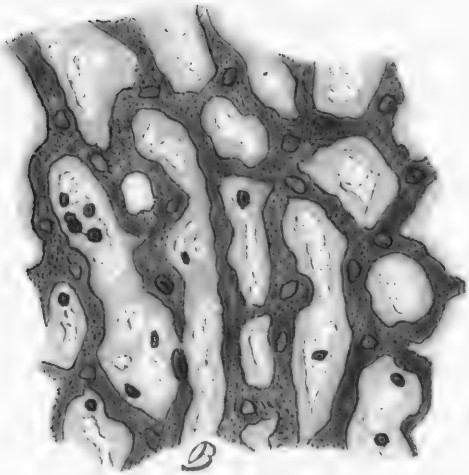


FIG. 2.

(Bailey: Amyloid Disease and Chronic Nephritis.)

FIG. 3.

(Bailey: Amyloid Disease and Chronic Nephritis.)

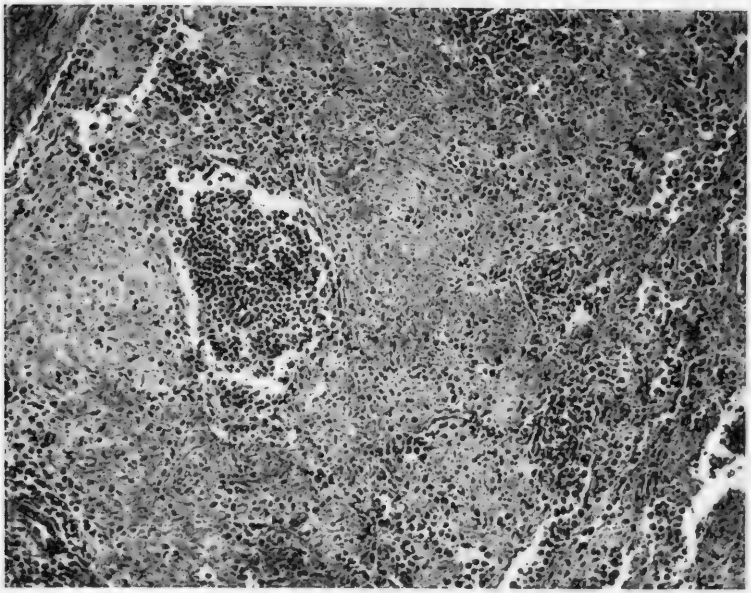


FIG. 4.

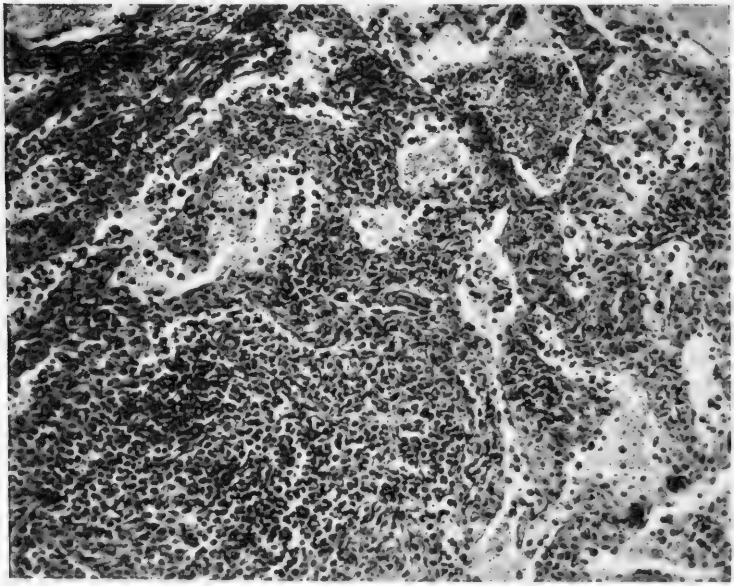


FIG. 5.

(Bailey: Amyloid Disease and Chronic Nephritis.)

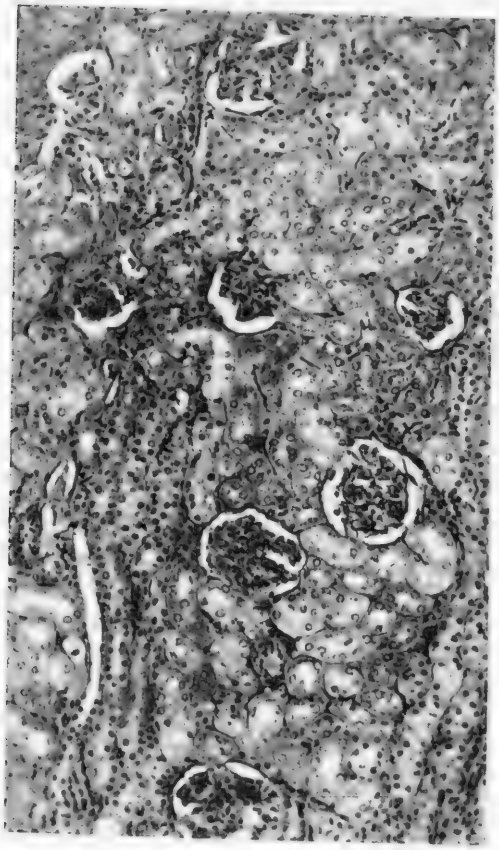


FIG. 6.

(Bailey: Amyloid Disease and Chronic Nephritis.)

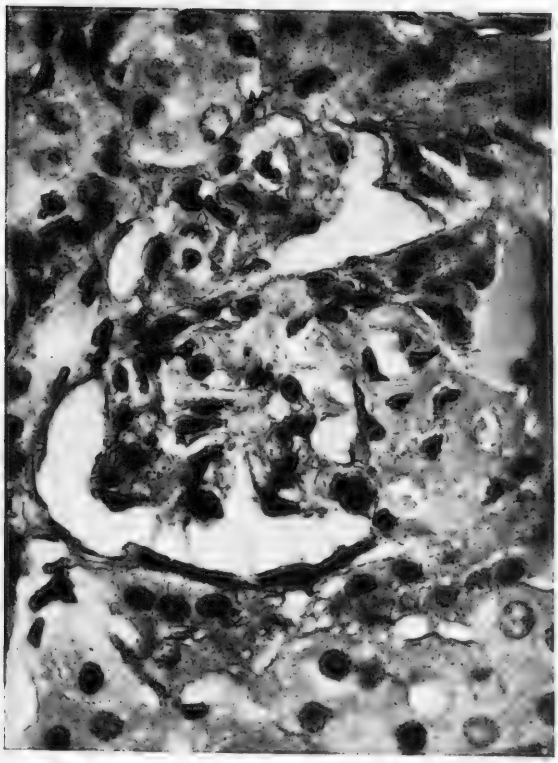


FIG. 7.

(Bailey: Amyloid Disease and Chronic Nephritis.)

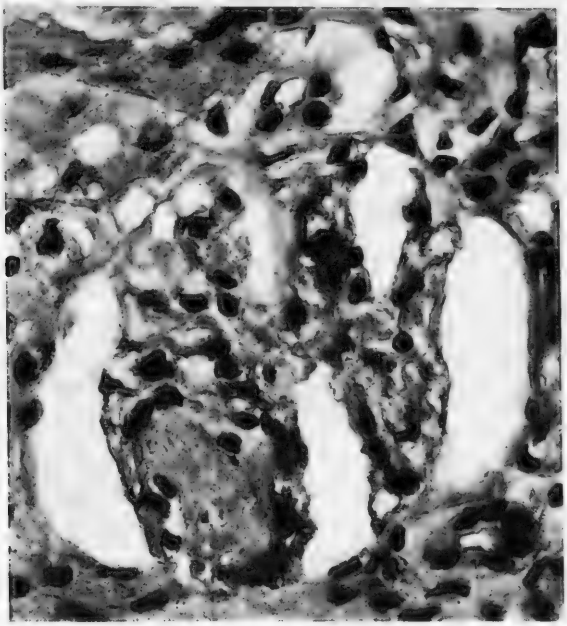


FIG. 8.

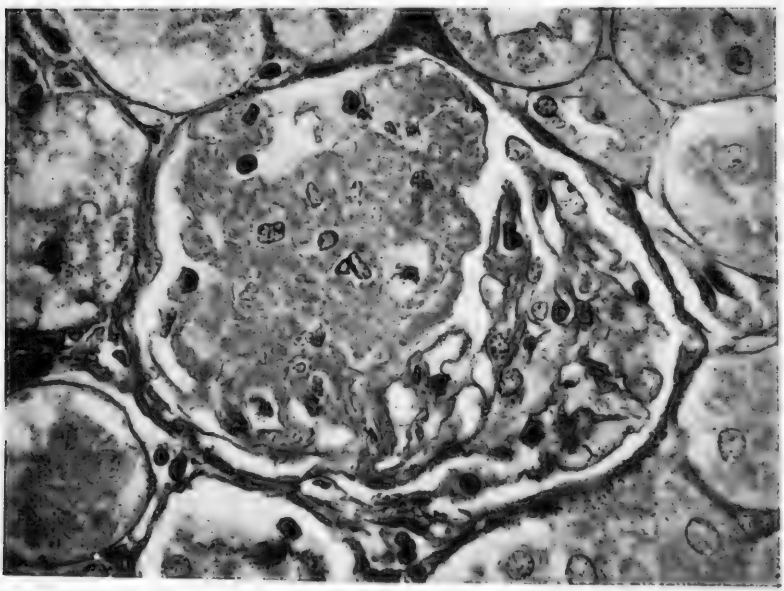


FIG. 9.

(Bailey: Amyloid Disease and Chronic Nephritis.)

# BACTERIOLOGICAL AND EXPERIMENTAL STUDIES ON GASTRIC ULCER.

By HERBERT L. CELLER, M.D., and WILLIAM THALHIMER, M.D.,*

(*From the Pathological Laboratory, Mount Sinai Hospital, New York.*)

(Received for publication, March 8, 1916.)

Many investigators have attempted to produce chronic gastric ulcers experimentally. These experiments have been performed on various species of animals, and a great variety of methods has been used in causing the original injury to, or defect in the gastric mucosa. All these attempts have failed, and the continuity of the gastric mucosa was always restored by prompt healing unless the procedure caused a complete stoppage of the blood supply to a part of the stomach. This was followed in some instances by infarction and by perforation and its sequelæ.

Recently Rosenow[1] has published some interesting investigations on the production of acute and chronic gastric ulcers in rabbits and dogs. His full report has not yet appeared. He isolated anhemolytic streptococci, so called *Streptococcus viridans* or *mitis*, from 96 per cent of a series of gastric ulcers removed from human subjects at operation. Recently isolated cultures were injected intravenously into animals, and in 60 per cent gastric ulcers were found. In a few animals which were allowed to live for a considerable time after their inoculation, in some instances several months, chronic ulcers were found at autopsy. From these results Rosenow has reached certain conclusions which may be briefly summarized as follows:

1. Anhemolytic streptococci can be recovered by a special technique from practically all gastric ulcers removed at operation.

2. The streptococci from this source possess a specific affinity for the stomach which enables them to localize in this organ, when recently isolated cultures are injected intravenously into animals.

3. About 60 per cent of the animals inoculated in this manner develop gastric

* This work was done under the tenure of a George Blumenthal, Jr., Fellowship.

[1] Rosenow, E. C., and Sanford, A. H., *Jour. Infect. Dis.*, 1915, xvii, 219; *Jour. Ind. State Med. Assn.*, 1915, viii, 458; *Jour. Am. Med. Assn.*, 1915, lxv, 1687.

ulcers. Other lesions also occur following these inoculations, but with less frequency.

4. Streptococci can be recovered from these experimental ulcers and can be demonstrated histologically. They reach this location by the blood stream and are deposited in the capillaries of the gastric mucosa.

5. These streptococci are identical with those inoculated.

6. Anhemolytic streptococci are, therefore, the cause of gastric ulcers in man, and these organisms reach the stomach by a hematogenous route.

The following experiments were undertaken following Dr. Rosenow's presentation of his investigations in New York in April, 1915. The technique which we employed in isolating and injecting the streptococci is identical with his. We are indebted to Dr. Rosenow for having furnished us with a description of his technique before the publication of his work.

*Material.*

Eight chronic gastric ulcers removed at operation from human subjects were examined bacteriologically and histologically We have also studied one ulcer occurring at the ostium of a gastrojejunostomy. The results obtained from these examinations and the animal experiments performed with the organisms recovered from these ulcers form the basis of the following report. Although the number of specimens studied has not been large, the consistency of the results obtained by all three methods of investigation seems to us to justify the present communication.

The eight gastric ulcers studied were all of the chronic indurated type. All the cases from which they were removed had typical gastric symptoms which varied in duration from 2 months to 8 years. Two of the ulcers were located at the pylorus and six on the lesser curvature.

On examination of the stomach at operation the ulcer-bearing area was found thickened and indurated and either a defect in the mucosa or a crateriform ulcer was felt.

Brief descriptions of the gross appearance of the specimens examined follow.

A. Small piece of ulcer removed. Ulcer felt crateriform to operator.

C. Funnel-shaped ulcer of the greater curvature, near pylorus, 1.5 cm. in width, with firm fibrotic margins.

D. Ulcer of the lesser curvature, 1.5 cm. in width, extending through the entire gastric wall into the gastrohepatic omentum. Edges overhanging, firm, and fibrotic.

E. The same appearance as D.

G. Ramifying, irregular ulcer involving the lesser curvature, the entire pyloric antrum, and the first cm. of the duodenum. It has a punched out appearance with sharp edges, not overhanging; the base extends into the muscularis.

J. At the middle of the lesser curvature of the pyloric antrum there is an ulcer 2 cm. in width, perforating entirely through the stomach wall into the gastrohepatic omentum, with sharp, firm edges that do not overhang.

L. Round, punched out ulcer, 1 cm. in width, with firm edges which slightly overhang. The ulcer extends almost through the entire wall of the stomach.

M. The specimen consists of a segment of the middle 7 cm. of the stomach. The middle of the posterior wall is occupied by a perforated ulcer, 3 cm. in diameter, with sharp edges.

*Gastrojejunal Ulcer.*—This specimen was removed from a patient on whom 3 years previously a posterior gastrojejunostomy by the Murphy button method had been performed, together with a pyloric exclusion by the string method for a perforated duodenal ulcer situated just beyond the pylorus.

*Operation and Specimen.*—At the site of the former duodenal ulcer there is an indurated area. The string is still in place and the pylorus almost completely closed, barely admitting the tip of the little finger. The gastrojejunostomy opening is considerably stenosed and admits only the tip of the little finger. The edge of the ostium appears soft and normal over about two-thirds of its circumference. The remaining one-third feels indurated and the serous surface over this is bright red in color and shows a few very recent, fibrinous adhesions between the jejunum and the transverse mesocolon. The anastomosis between the jejunum and the stomach was separated with difficulty. Beneath the reddened portion of the ostium was found what appeared to be a definite and somewhat indurated ulcer of the stomach, 1.5 cm. in size. This portion was resected and a new gastrojejunostomy was performed at the same site, by the suture method.

## *Methods.*

Immediately after the operation pieces of ulcer were removed for culture and the remainder of the material was fixed in 70 per cent alcohol. Some of the pieces were incubated in deep tubes of glucose-serum-bouillon for 24 and 48 hours and then fixed in alcohol. Sections were prepared by both the paraffin and celloidin methods and stained with hematoxylin and eosin, Giemsa and Unna-Pappenheim stains, and by the Gram-Weigert method.

Formalin fixation was not used as this interferes with the demonstration of bacteria. The aniline oil, gentian violet used in the Gram-

Weigert method was always filtered through Berkefeld filters to remove any bacteria present which might be deposited on the section and lead to confusion.

*Microscopical Studies.*

Microscopical examination reveals a great similarity in the pathological processes in the specimens. All the ulcers show certain prominent characteristics. The edge of the ulcer is sharp, as though cut off, even when it is overhanging. The mucosa at the edge shows only a superficial degeneration and a moderate grade of chronic inflammation consisting of congestion, edema, and diffuse infiltration with mononuclear leukocytes, plasma cells, and a few polymorphonuclear leukocytes. The glandular structures at the edge of the ulcer show slight degenerative changes of their cells. Some of the glandular acini or crypts also have the appearance of being cut off and do not reach the surface of the mucosa but terminate at the edge of the ulcer. Whenever found, the cells of the acini are contained within their basement membrane and show no tendency to hyperplasia or malignant transformation. The lining, *i. e.*, the surface of the ulcer, consists of a narrow layer from one-eighth to one-third the diameter of a low power field of the microscope in thickness and resembles very much the degenerating tissue which is found situated in tuberculous lesions between the areas of caseation and the surrounding tuberculous granulation tissue. The layer of degenerating tissue fuses with or is transformed into dense fibrous tissue, although the line of demarcation is clearly seen. The fibrous tissue extends for a considerable distance, in places as much as 1 cm., into the submucosa and muscularis. It has the typical characteristics of scar tissue and seems to form a wall beneath the ulcer. Some of the ulcers have completely penetrated the stomach wall, but the edges are always covered by the above structures and the bare muscularis is never exposed. The connective tissue septa which lie between the muscle bundles and are accompanied by blood vessels, are increased in size by fibrous tissue and dense infiltration with mononuclear leukocytes. Similar areas of fibrosis and infiltration are found beneath and in the serosa.

Occasionally, but only rarely, prolongations of the degenerat-

ing surface layer, several mm. in size, are found in the dense fibrous tissue layer, but these are always completely limited by the latter.

With the special stains for bacteria, especially with the Gram-Weigert stain, organisms are found only in or upon the lining of the degenerating tissue but never in the depths of this except in the prolongations described above. The organisms usually occur in small clumps or as sparsely scattered individuals, and are not found in the dense fibrous layer, the stomach wall, the connective tissue septa, or the areas of leukocytic infiltration of the serosa. The chief organisms found are minute cocci (streptococci), peculiar forms of yeast, and various types of bacilli. The same forms are found in great numbers throughout the tissue which was incubated in bouillon before it was fixed in alcohol, but are most numerous on and near the surface of the ulcer. The types of organisms observed in the preparations from the material fixed immediately and the organisms recovered in cultures are given in parallel columns in Table I.

In all, 109 tubes were inoculated either with emulsions or pieces of ulcer. Streptococci were present in 32 tubes, occurring in pure culture in 11. 13 tubes remained sterile, and the other 64 tubes contained various types of organisms.

The streptococci were anhemolytic and had the following morphological and cultural characteristics.

*Morphology.*—Stained preparations. Small, Gram-positive cocci, forming short and long chains. No capsule. Hanging drop of growth in deep tubes of glucose-serum-bouillon shows long convoluted chains.

*Bouillon.*—Slight diffuse cloudiness; many granular clumps adherent to the sides of the tube and also deposited at the bottom as sediment.

*Litmus Milk.*—Acidified but not coagulated.

*Gelatin.*—Not fluidified.

*Inulin-Serum-Peptone-Water-Medium.*—Not fermented.

*Bile.*—No solution of organisms.

*2 Per Cent Glucose-Serum-Agar.*—Dry, granular, fairly profuse growth. All strains cause precipitation, most of them marked precipitation.[2]

*Plain Agar.*—Sparse, dry growth, mostly as minute, discrete, pearly white colonies.

---

[2] Libman, E., *Jour. Med. Research*, 1901, i, 84.

TABLE I.

| Ulcer. | Types of organisms found in sections of ulcers. | Organisms recovered in cultures from ulcers. |
|---|---|---|
| A | Few groups of round bodies, suggestive of cocci, upon surface of ulcer. | Streptococci and several unidentified bacilli. No pure cultures. 6 tubes inoculated; 2 contained streptococci. |
| C | Small groups of cocci, few bacilli, some of them diphtheroids, found in and on degenerating lining near edge of ulcer. | Streptococci, *Bacillus subtilis*, *Micrococcus tetragenus*, large Gram-positive bacilli, diphtheroid bacilli. 8 tubes inoculated. Streptococci pure in 1 and together with bacilli in another. |
| D | Few clumps of 15 to 20 small cocci on surface of ulcer. | Streptococci, yeast, *Staphylococcus albus*, Gram-positive and Gram-negative motile bacilli, Gram-positive non-motile bacilli. 14 tubes inoculated, 2 of which contained pieces of ulcer. In one of the latter streptococci were found in pure culture; in 1 other tube they occurred together with Gram-positive bacilli. |
| E | Large numbers of organisms singly and in clumps found on degenerating surface of lining of ulcer. These are mostly small cocci in long chains; many large bacilli and a few slender bacilli. | Streptococci were seen in cultures but could not be isolated; slender Gram-positive bacilli, staphylococci, spore-bearing Gram-positive bacilli, and a pleomorphic Gram-positive bacillus were isolated. 13 tubes inoculated, 2 of which contained pieces of ulcer. In the latter cocci in long chains were seen; 4 tubes were sterile. |
| G | Few organisms in and upon degenerating lining of ulcer. These are mainly large and small bacilli and a few cocci. | Streptococci, Gram-positive bacilli, motile Gram-negative bacilli, *Staphylococcus albus*, *Bacillus subtilis*, Gram-negative spore-bearing bacilli. 14 tubes inoculated, 2 of which contained pieces of ulcer. In 1 of the latter streptococci were found in almost pure culture and in the other there were a few chains of streptococci and Gram-negative motile bacilli.<br>Of the remaining 12 tubes, 1 contained streptococci in pure culture, and 4 contained streptococci together with 1 or more other organisms. |

TABLE I—*Concluded.*

| Ulcer. | Types of organisms found in sections of ulcers. | Organisms recovered in cultures from ulcers. |
|---|---|---|
| J | Few bacilli on surface of ulcer. In a prolongation of the necrotic lining of the ulcer into the depth there are yeasts, scattered and in large clumps; small cocci, some of which are in chains; large cocci. | Streptococci, yeast, staphylococci, *Bacillus subtilis*, spore-bearing Gram-negative bacilli. 16 tubes inoculated, 2 with pieces of ulcer, which contained streptococci together with Gram-positive bacilli. In 3 of the remaining tubes streptococci were found in pure culture, in 4 together with other organisms, and 1 tube was sterile. |
| L | In and upon the degenerating lining are found masses of yeast but no bacteria. | Yeast, *Staphylococcus albus*, small Gram-positive bacillus. 12 tubes inoculated. None contained streptococci, but yeast and *Staphylococcus albus* were recovered from all. |
| M | In and upon the degenerating lining are many cocci, some in chains. There are as many bacilli present as cocci; some of the bacilli are large and in chains and some are small. Yeasts in tube-like stems are also found. | Streptococci, yeast, *Bacillus subtilis*, staphylococci, Gram-positive bacilli, large streptobacilli. 15 tubes inoculated, 2 with pieces of ulcer. The latter contained streptococci together with other organisms. Of the remainder, 2 contained streptococci in pure culture, 1 contained streptococci and bacilli, and 7 were sterile. |
| Gastrojejunal. | No bacteria demonstrable by Gram-Weigert method. | Streptococci, staphylococci, short Gram-positive bacillus. 11 tubes inoculated, 1 with piece of ulcer. Streptococci recovered in pure culture from 3 tubes and together with staphylococci in 2 tubes. 1 tube was sterile, 1 contained a short Gram-positive bacillus, and 4, including the tube inoculated with a piece of ulcer, contained staphylococci. |

*Plain Blood Agar.*—Small discrete or fused colonies, gray by reflected light, and opaque and surrounded by a narrow green zone by transmitted light. No hemolysis.

From seven ulcers we recovered anhemolytic streptococci; streptococci were seen in cultures from the eighth but could not be isolated, and from the ninth no streptococci were recovered.

Yeasts were recovered from four ulcers. They were not identified, but grew luxuriantly aerobically on all the ordinary laboratory media. Morphologically slight variations in the strains were noted. They resembled morphologically the Saccharomyces described by Besson.[3]

*Method of Inoculation.*

Recently isolated cultures were injected in most of the experiments, the exact generation being given in the table, "first generation" meaning the original culture. The material for inoculation was prepared as follows:

1. A 24 hour growth of streptococci was obtained either in deep tubes of glucose-serum-bouillon or on slants of glucose-serum-agar. About 20 cc. of the growth in bouillon were centrifuged, washed twice with normal saline solution, and the resulting sediment was taken up in 2 to 5 cc. of normal saline solution. The 24 hour growth from one to four glucose-serum-agar slants was similarly washed with normal saline solution and the sediment resuspended in salt solution.

2. A milky emulsion of a 24 hour culture of yeast on glucose-serum-agar was made in several cc. of normal saline solution.

The entire amount of suspension prepared was used for intravenous injection into the ear vein and from 1 to 2 cc. for injection into a branch of the gastric artery. For the gastric artery injection the animals were etherized and a laparotomy was performed under aseptic precautions. The stomach was delivered and a branch of the gastric artery, usually on the anterior wall, was injected with a very fine needle and Record syringe. The part supplied always blanched during injection, but the color immediately returned on withdrawal of the needle. Very little hemorrhage followed and this was quickly controlled by compression. The wounds in every animal healed *per primam* and none developed peritonitis. Subsequent investigation was made through a laparotomy wound, opening the stomach widely and inspecting the entire mucosa directly. These gastric incisions also healed promptly and in several animals the stomach was opened a second time without apparent injury.

[3] Besson, A., Technique microbiologique et sérothérapique, Paris, 5th edition, 1911.

### *Animal Experiments.*

Rabbits and cats were used in our experiments. Most of the rabbits were injected intravenously with streptococci, but in some the injections of either streptococci or yeast were made directly into a branch of the gastric artery. Streptococci were injected into a branch of the gastric artery in two cats and a yeast in the other two.

Table II gives in a condensed form the experiments and their results.[4]

### *Gastric Lesions in Rabbits Following Intravenous Injection of Streptococci.*

Thirty rabbits were injected intravenously with streptococci. Gastric lesions developed in four (13.3 per cent).

*Rabbit A III.*—Killed 2 days after injection.

*Stomach.*—Over the anterior wall of the stomach near the cardia there are three pale, almost circular areas seen on the serosa. One of these is at the termination of a group of blood vessels springing from the lesser curvature. Beneath these areas are what appear to be defects in the mucosa, from 2 to 10 mm. in width. The edges are abrupt and irregular and one cannot tell whether the base of the defects is covered by the mucosa or not.

*Microscopical Examination.*—The defects extend almost through the mucosa, with slight inflammatory reaction about them. Gram-Weigert stain shows various types of bacilli and cocci along the entire surface of the mucosa and defect. The same organisms occur in the tissues just beneath the surface, but at no greater depth at the site of the defect than elsewhere. No predominance of cocci.

*Rabbit C III.*—Killed 1 day after injection.

*Stomach.*—The anterior wall of the cardiac region is bright red. The mucosa beneath this is also bright red. No defects found.

*Microscopical Examination.*—(Two blocks.) Section 1. From non-hemorrhagic area. Tissues are normal. There are no bacteria in the mucosa, but on the surface are found the large Gram-positive bacilli that occur on the surface of all stomach sections.

Section 2. From hemorrhagic area. There is an engorgement of the capillaries in the mucosa just beneath the surface, and slight extravasation. No bacteria in this area. At the other end of the section, which includes normal tissue, just beneath the surface of the mucosa, there is a very large clump of Gram-positive cocci, some of them in chains, but mostly occurring as diplococci or

[4] A number of experiments were performed with organisms recovered from other gastric ulcer cases, which we hope to embody in a later report.

TABLE II.

*Stomach Lesions.*

| Ulcer. | Rabbit. | Days animal lived after injection. | Generation of streptococcus or yeast injected. | Intravenous injection. | Gastric artery injection. | After intravenous injection. | After gastric artery injection. | Heart lesions. | Cultures of urine, bile, and heart's blood. |
|---|---|---|---|---|---|---|---|---|---|
| | | | Streptococcus. | | | | | | |
| A | I | 1 | 2 | + | | | | | |
| | II | 4 | 2 | + | | | | Few hemorrhages. | Negative. |
| | III | 2 | 2 | + | | Defect. | | Hemorrhages. | |
| | IV | 10 | 2 | + | | | | | " |
| C | I | 2 | 3 | + | | | | Endocardial hemorrhages. | |
| | II | 3 | 3 | + | | | | Hemorrhages. | Streptococci from blood; urine and bile negative.* |
| | III | 1 (Killed.) | 4 | + | | Hemorrhages. | | " | Streptococci and staphylococci in blood; urine and bile negative.† |
| | IV | 3 | 4 | | + | | Abscesses and small defects. | Gray streak in septum. | |
| | II 1 | 3 | 3 (From Rabbit C II.) | + | | | | Pink and gray nodules in endocardium, epicardium, and valves. | Negative. |
| | II 2 | 6 | 3 (From Rabbit C II.) | + | | | | | " |
| D | I | 5 | 1 | + | | | | | Streptococci from heart's blood. |
| | II | 3 | 1 | + | | | | | Negative. |
| | III | 2 | 3 | + | | | | Gray streak in septum. | Streptococci from blood and urine.‡ |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | IV | 4 | 3 | + | | | | Gray streaks in heart. | |
| E | I | Died immediately.§ | Original bouillon culture. Streptococci seen; not isolated. | + | | | | | |
| | II | 1 (Died.) | | + | | | | Cocci found microscopically in blood vessels; no lesions.‖ | |
| | III | 1 " | | + | | Reddened; no hemorrhages. | | | Staphylococci and bacilli from heart's blood.** |
| G | I | 2 | 1 | + | | | | Hemorrhages. | Negative. |
| | II | 7 | 1 | + | | | | | " |
| | III | 7 | 1 | + | | | | | " |
| | IV | 2 | 2 | + | | | | | " |
| | V | 14 | 2 | + | | | | | " |
| | VI | 3 | 2 | + | | Hemorrhage and shallow defect. | | Mottled appearance. | Streptococci from heart's blood.* |
| | VII | 5 | 3 | | + | | Defect. | | Negative. |
| | VIII | 4 (Died.) | 3 | | + | | Minute hemorrhages. | | |
| J | I | 7 | 1 | + | | | | | " |
| | II | 4 | 1 | + | | | | | " |
| | III | 7 | 4 | | + | | Defect. | | " |
| | IV | 4 | 4 | + | | | | | " |
| | V | 7 | 4 | | + | | | | |
| | VI | 7 | 4 | + | | | | Hemorrhage in tricuspid valve. | " |

* Gray streaks in skeletal muscles. † Hemorrhages in intestines and appendix. ‡ Tissues throughout body reddened. § Died with symptoms of anaphylactic shock. ‖ Hemorrhages in intestines. ** Tissues congested.

TABLE II—*Concluded.*

| Ulcer. | Rabbit. | Days animal lived after injection. | Generation of streptococcus or yeast injected. | Intravenous injection. | Gastric artery injection. | After intravenous injection. | After gastric artery injection. | Heart lesions. | Cultures of urine, bile, and heart's blood. |
|---|---|---|---|---|---|---|---|---|---|
| | | | Streptococcus. | | | | | | |
| M | I | 7 | 1 | | + | | Defect. | | Negative. |
| | II | 16 | 1 | | + | | | | |
| | III | 7 | 1 (Killed culture.) | | + | | Minute hemorrhages. | | |
| | IV | 2 | 1 | + | | | | Hemorrhages. | Streptococci from heart's blood. |
| | V | 11 | 1 | + | | | | | |
| Gastro-jejunal ulcer. | I | 2 | 3 | + | | | | | |
| | II | 5 | 3 | + | | | | | |
| Summary | 38 | | | 30 | 8 | 4 (13.3 per cent.) | 6 (75 per cent.) | 16 | |
| | | | Yeast. | | | | | | |
| L | I | 5 | 1 | + | | | | | |
| | II | 7 | 3 | | + | | | | |
| | III | 1 | 3 | + | | Hemorrhage. | | Hemorrhages. | |
| | IV | 7 | 3 | + | | " | | | |
| Summary | 4 | | | 3 | 1 | 2 (66 per cent.) | 0 | 1 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cat | | Streptococcus. | | | | | | |
| J | I | 33 | 8 | | + | | Small defect which healed. | | |
| | II | 33 | 8 | | + | | Large defect which healed. | | |
| Summary | 2 | | | | 2 | | 2 (100 per cent.) | | |
| | | | Yeast. | | | | | | |
| L | I | 12 | 4 | | + | | Negative. | | |
| M | I | 13 | 2 | | + | | " | | |
| Summary | 2 | | | | 2 | | 0 | | |

as bacillary forms. There is no inflammatory reaction found around this area and only a slight, almost insignificant amount of extravasation of red blood cells.

*Rabbit G IV.*—Killed 2 days after injection.

*Stomach.*—On the anterior wall of the pyloric antrum, near the lesser curvature are two hemorrhagic areas in the mucosa, 2 by 4 mm. in size, and a few mm. from one another. There is a superficial defect over these areas and the mucous membrane around them for a distance of 2 mm. is slightly reddened.

*Microscopical Examination*—Serial sections of superficial defect. Gram-Weigert stain. A V-shaped defect, half way through the mucosa, in the midst of the hemorrhagic area. Very large bacilli are invading the defect from the surface. No streptococci. No bacteria in any of the blood vessels.

*Rabbit G VI.*—Animal became sick and was killed 3 days after injection.

*Stomach.*—On the mucosa on the anterior wall of the pyloric antrum there is a superficial hemorrhagic defect, 2 by 4 mm. The mucosa around it is reddened, and adherent to it is blood-stained mucus.

*Microscopical Examination.*—A considerable area of mucosa shows slight hemorrhagic infiltration, with hyaline thrombosis in a few of the superficial blood vessels and moderate degeneration of the superficial third of the mucosa. Just beneath the center of this area are several arteries and veins in the submucosa which show slight inflammation of their walls but no change in the lumen.

*Serial Sections.*—Gram-Weigert stain. No cocci in any of the vessels going to the mucosa. No bacteria except surface bacteria of various types.

### *Gastric Lesions in Rabbits Following the Injection of a Branch of the Gastric Artery with Streptococci.*

In eight rabbits a branch of the gastric artery was injected with streptococci, according to the method described above. Gastric lesions developed in six (75 per cent).

*Rabbit C IV.*—Killed 3 days after injection.

The peritoneal cavity contains a small amount of fluid and clotted blood, and the omentum and portions of the stomach are infiltrated with blood. Omentum adherent over stomach. Serosa over injected area is pale, and shows beneath it a few pin-head, cream-colored areas, which look like miliary abscesses. Mucosa over these areas shows small hemorrhagic patches with slight defects in the mucosa, as well as miliary, cream-colored areas similar to the above.

*Microscopical Examination.*—There are several large hemorrhagic areas in the serosa and collections of leukocytes almost to the degree of abscess formation. There are about half a dozen depressions along the surface of the mucosa which are V-shaped, and about these depressions is a slight hemorrhagic infiltration with partial degeneration (?) of the structures. These areas reach half way through the mucosa. Over most of these depressions the surface epithelium is

intact but slightly degenerated and there is a slight fibrinous exudate adherent to the surface.

Gram-Weigert stain. Upon the mucosa over the hemorrhagic and purulent areas are a great number of bacteria, most of them large bacilli; no bacteria are found in the hemorrhagic and purulent areas in the mucosa. Bacteria are present in the serosa and correspond to those on the surface of the stomach. These are probably secondary invaders.

*Rabbit G VII.*—Killed 5 days after injection.

*Stomach.*—The branch of artery injected is identified by a small hemorrhagic spot at the site of injection. Distal to this the vessel seems empty. On the serous coat, in the portion directly supplied by the injected artery, an area, 1.5 by 3 cm., shows beneath the serosa a thin layer of thick, cream-colored pus. Beneath this is seen, from the mucosal side, an irregular, punched out defect, 1 by 0.5 cm., with raised, edematous edges, and an area of edema around it, 2 cm. in width. The base of the defect is formed by a very thin, transparent portion of the wall of the stomach. About 2 cm. from the defect is a hemorrhage in the mucosa, 2 mm. wide. The remainder of the stomach is normal.

*Microscopical Examination.*—The base of the defect shows degeneration and necrosis almost as far as the serosa. The edge of the defect is fairly sharp and shows a similar condition of degeneration.

Gram-Weigert stain. In the depth of the defect, just beneath the serosa and reaching for a short distance into the muscularis, are found solid clumps of streptococci filling the capillaries and small blood vessels. Between these, scattered throughout the tissues, are diffuse collections of streptococci. On and immediately beneath the necrotic surface of the defect are large clumps of bacilli. Individual bacilli also lie scattered between the clumps.

On cross section of a portion of the stomach with arteries near the defect, one of the arteries is seen to be filled with an organizing thrombus; the other arteries are normal. There are no bacteria in the thrombus.

*Rabbit G VIII.*—Died 4 days after injection.

*Stomach.*—About one-third filled with food. Delicate adhesions between the cardiac half of stomach and surrounding viscera. Over the cardiac half of the stomach, especially near the greater curvature, the mucosa shows numerous minute hemorrhages. No defects.

Microscopical examination was not made.

*Rabbit J III.*—Killed 7 days after injection.

*Operation.*—Attempt to make injection into a branch of the gastric artery near the cardiac end of the stomach on the anterior wall failed. Injection was made into another blood vessel on the anterior wall of the stomach, though it could not be determined whether it was a vein or an artery. No blanching of the portion of the stomach injected. Previous attempt at injection resulted in a large hematoma at the lesser curvature of the stomach. Two large branches of the gastric artery on the posterior wall of the stomach were injured in delivery of the stomach. Animal returned to cage in good condition.

*Stomach.*—Hematoma along the lesser curvature. About the vessel on the anterior wall which was injected there is an area about 1 by 0.5 cm. showing hemorrhages and a small amount of thick pus beneath the serosa. On the posterior wall of the fundus near the greater curvature, corresponding to the area supplied by the injured arteries, the stomach is adherent in two places to one of the lobes of the liver. The mucosa of the stomach immediately beneath these adherent areas shows an irregular defect 0.5 cm. in width which extends half way down through the gastric wall. The base of the defect is reddened; the edge is edematous and slightly reddened. Beneath the hemorrhagic areas, on the anterior wall of the stomach where the injection was made, there are no changes in the mucosa. The stomach is otherwise negative.

*Microscopical Examination.*—Defect on posterior wall. Superficial defect half way through the mucosa with degenerative changes, slight hemorrhages, etc. One large vein in the submucosa shows beginning mural thrombosis. No streptococci or other bacteria in the depth of the defect. Various types of bacteria present on the surface of the normal portion of the stomach and on the surface of the defect. Adhesions over the serosa contain no bacteria. Gastric arteries supplying the area of the defect are normal.

Gram-Weigert stain. Usual types of bacteria on surface of defect and neighboring normal mucosa, none in tissues except a few surface type organisms in the hemorrhagic area of the serosa.

The serosa of lesions on the anterior wall shows edema and slight hemorrhagic and leukocytic infiltration. Vessels and mucosa are normal. Gram-Weigert stain. Usual surface bacteria are present, but no organisms are found in the tissues.

*Rabbit M I.*—Killed 7 days after injection.

*Stomach.*—On the serous surface of the anterior wall of the fundus, in the center of the region supplied by the injected artery, there is a creamy yellow area, 1 by 2 cm., where the omentum is adherent. There are other smaller areas of thickening of serosa. Beneath the large area, practically the entire thickness of the stomach wall from the mucosa outward is necrotic, forming a defect with hemorrhagic edge and necrotic base. The branch of artery injected is in good condition.

*Microscopical Examination.*—There is a defect of the mucosa. Beneath the defect the gastric wall shows large masses of pus cells, edema, congestion, and degeneration of tissues, amounting almost to necrosis in places. No thrombi in veins or arteries.

Gram-Weigert stain. There are masses of cocci in the inflamed and degenerating area and similar masses in the overhanging edge of the defect apparently lying in the capillaries or lymphatics.

*Rabbit M III.*—Killed 7 days after injection with killed culture of streptococci.

*Stomach.*—Minute scar at the point of injection of the artery. Mucosa of fundus, near the greater curvature, shows a few minute hemorrhages but no defect over them.

*Microscopical Examination.*—Congestion of superficial capillaries. No hemorrhages. Gram-Weigert stain. No cocci, or other bacteria, except a few usual types on the surface.

### *Gastric Lesions in Cats Following the Injection of a Branch of the Gastric Artery with Streptococci.*

In two cats a branch of the gastric artery was injected with streptococci. Both animals developed defects in the gastric mucosa, which soon began to heal and were observed to have healed completely in 33 days.

*Cat J I.*—Cat well 9 days after injection.

*Stomach Operation.*—Explored under ether anesthesia. Few adhesions found between the anterior wall of the stomach and the omentum, and between the posterior wall and the posterior wall of the lesser peritoneal sac. Along the lesser curvature there is an indurated area about 1 cm. wide in the region supplied by the injured artery. Beneath this is seen a punched out area in the mucosa that may be either a shallow erosion or healing defect. Stomach otherwise negative. The incision in the stomach, which was parallel to the greater curvature and 1 cm. from it on the anterior wall, was closed with a double row of sutures. 33 days after injection the abdomen was opened under ether anesthesia. No sign of defect found on serous or mucous coats of stomach. Animal killed.

*Cat J II.*—18 days after injection. Cat has remained entirely well and well nourished.

*Operation.*—Performed under ether anesthesia. Stomach free and nowhere adherent. At the lesser curvature, where the injected artery enters the stomach, there is an indurated area involving the entire wall of the stomach, 1.5 cm. wide and about the normal stomach wall in thickness. The stomach was opened on the anterior wall parallel to the greater curvature for a distance of 4 cm., 1.5 cm. from the greater curvature and the same distance from the indurated area. Beneath this indurated area is a shallow, irregularly round defect in the mucosa with indurated margins. The edge of the defect is formed by a bright red line, apparently of granulations, as though it were healing. Stomach closed. Animal returned to cage in good condition. 33 days after the injection the cat was again etherized. Slight puckered scar of serosa over the site of the previous defect. Mucosa at corresponding point shows slightly puckered area, entirely healed. Animal killed.

*Microscopical Examination.*—The mucosa is completely regenerated over the site of the ulcer. It is made up of glands which are slightly atypical. A slight defect in the submucosa has also been repaired. Gram-Weigert stain shows no bacteria.

*Gastric Lesions in Rabbits Following Injection with Yeast.*

Three rabbits were injected intravenously. Two of these animals developed minute hemorrhages in the gastric mucosa.

*Rabbit L III.*—Died 12 hours after injection.
*Stomach.*—Many minute hemorrhages in mucosa.
*Microscopical Examination.*—Negative; no yeast.
*Rabbit L IV.*—Killed 7 days after injection.
*Stomach.*—Few minute hemorrhages in mucosa, not examined microscopically.

In addition a branch of the gastric artery was injected in one rabbit (L II). The animal was killed on the 7th day and showed no lesions at autopsy.

*Gastric Lesions in Cats Following Injection of Yeast into a Branch of the Gastric Artery.*

Two cats (L I, M I) were injected. The animals died on the 12th and 13th days respectively, and no lesions were found at autopsy.

*Summary of Cardiac Lesions.*

Of the thirty rabbits injected intravenously with streptococci, fourteen (47 per cent) developed cardiac lesions. In two, minute vegetations containing streptococci were present. The remainder showed either petechial hemorrhages or the gross appearance of myocarditis, and practically all of these hearts contained microscopical lesions of the Bracht and Wachter type.[5]

Of the eight rabbits in which a branch of the gastric artery was injected two (25 per cent) developed minute hemorrhages in the endocardium.

Of the three rabbits injected intravenously with yeast one showed hemorrhages in the endocardium.

## DISCUSSION.

As a result of the above studies it is impossible to decide definitely whether or not the gastric lesions produced by the injection of streptococci are to be considered ulcers. The superficiality of the rabbit lesions following the injection by the intravenous route as well as the entire absence of inflammatory reaction in the deeper gastric

[5] Thalhimer, W., and Rothschild, M. A., *Jour. Exper. Med.*, 1914, xix, 429.

tissues leads us to the belief that these defects are certainly not analogous to the chronic gastric ulcer seen in the human stomach. The promptness of healing of the embolic lesions in the cats tends to strengthen this conclusion. This point will be discussed more fully below.

Apparently striking results were obtained in Cats J I and J II, where the injection of streptococci into a branch of the gastric artery resulted in the formation of shallow, indurated defects that might be considered ulcers. However, these defects were found to be healing on the 10th and 18th days respectively, and were completely healed on the 33rd day.

It has been demonstrated by Bolton,[6] Wilensky and Geist,[7] and others that defects produced mechanically in the gastric mucosa and muscularis of cats heal in 2 to 4 weeks. In the defects produced in the rabbits by injection into a branch of the gastric artery streptococci were found in great numbers in the tissue about the lesions. By analogy it may be assumed, even in the absence of microscopic proof, that streptococci were also present at some time in the lesions in the cats. The embolic lesions in the stomachs of the cats in our series healed spontaneously within approximately the same length of time as those mechanically produced. It is evident, therefore, that in these instances the injected streptococci failed to retard the process of healing.

The constant presence of an anhemolytic streptococcus in human gastric ulcers might be adduced as an argument in favor of the part played by this organism as the cause of the chronicity of the lesion. If streptococci could be demonstrated in considerable numbers in the depth of human gastric ulcer this conception would gain a firmer basis. Rosenow[1] has found streptococci in all the coats of the stomach, some of the organisms even lying just beneath the serosa. No mention is made of the number of organisms in this position. In our series of ulcers, however, despite the examination of many sections, the streptococci were found only on or beneath the surface of the defect.

[6] Bolton, C., *Jour. Path. and Bacteriol.*, 1915, xx, 133.

[7] Wilensky, A. O., and Geist, S. H., *Jour. Am. Med. Assn.*, 1916, lxvi, 1382.

The case of gastrojejunal ulcer described above seems of importance in this connection. This ulcer might readily be considered as due primarily to purely mechanical factors. Streptococci were recovered culturally from emulsified pieces of tissue. Although the histological picture was identical with that of the other ulcers in the series the most careful search failed to show the presence of streptococci on the surface of the lesion or in the tissues about it. One must consider the possibility that in this case the streptococci were directly deposited upon a preexisting mechanical defect and were not the essential causative factor in the formation of the ulcer.[8]

A possible explanation of the occurrence of streptococci in gastric ulcers may be found in some observations of Cushing and Livingood.[9] These investigators found that whereas there is a more or less definite bacterial flora of the ileum and large intestine, this is not true of the upper portion of the alimentary tract. The stomach and the duodenum and at times also the upper jejunum become free or practically free of bacteria after a certain period of fasting, whereas during and shortly after a meal great numbers of organisms of different types are found. These are greatly reduced in number when sterile food alone is taken. Cushing and Livingood, therefore, concluded that the normal stomach has no definite bacterial flora but that this is dependent upon the bacteria taken with the food.

[8] Since this article was completed we have studied another gastrojejunal ulcer that had the gross and microscopic appearance of a chronic gastric ulcer. No bacteria were found with the Gram-Weigert stain either upon the surface of the lesion or in the underlying tissues. Anhemolytic streptococci were isolated from several tubes of glucose-serum-agar inoculated with the emulsified ulcer. Other bacteria similar to those described in the body of the paper were also recovered.

Six rabbits were injected intravenously with the second generation of the streptococcus and were killed at periods varying from 2 to 10 days. At autopsy there were small hemorrhages in the duodenal mucosa of one rabbit, hemorrhages in the tricuspid valve in two others, and minute tricuspid vegetations in a fourth. The structure of these vegetations on microscopical examination as well as the absence of streptococci in smears and sections indicates that the injected organisms were not the cause of the lesion. No other lesions were found in these four animals, and in the remaining two the autopsy was entirely negative. No streptococci were found either culturally or microscopically in the hemorrhagic area in the duodenum.

[9] Cushing, H., and Livingood, L. E., *Johns Hopkins Hosp. Rep.*, 1900, ix, 543.

Cushing and Livingood showed that neither the acidity nor the digestive ferments of the gastric secretion have marked lethal effect on the bacteria in the stomach. They believe that the stomach becomes aseptic chiefly through the mechanical removal of organisms in the passage of the ingesta, and that bacteria cannot adhere to normal gastric mucosa nor do they enter or remain in the glandular crypts. Whether bacteria can adhere to the surface or penetrate into the depths of defects in the mucosa remains to be determined. These writers further state that there were two organisms from which the stomach freed itself with difficulty, and which were found in a number of examinations many hours after the intake of food. These organisms were a minute Gram-positive diplococcus and yeast. The same forms were found almost constantly in our series of gastric ulcers both in sections and in cultures.

In the light of the work of Cushing and Livingood one must bear in mind the possibility that these organisms which are commonly found in the stomach and duodenum might remain upon or immediately beneath the degenerating lining of the ulcer because of favorable mechanical or structural conditions.

The nature of both the experimental gastric and cardiac lesions indicates that the streptococci recovered from the ulcers are of a low grade of pathogenicity, such as is usually found in the same type of organism isolated from other sources. Whether, having established themselves on the surface of a gastric ulcer in man, they are innocuous or manifest a low grade of pathogenicity tending to retard healing requires further investigation.

In addition to the streptococcus other organisms were invariably recovered from the inoculated tubes. No attempt was made to identify these strains culturally. The same types were usually identified microscopically in sections, proving that the isolated organisms other than streptococci were not accidental contaminations. Furthermore, these bacteria were quite as numerous in the sections as streptococci, and penetrated to the same depth in the ulcer. The types most frequently encountered were a yeast and a thick Gram-positive bacillus. Some of the isolated strains of yeast proved pathogenic for rabbits on intravenous inoculation. Our data are insufficient to warrant an expression of opinion as to the significance of these organisms.

## CONCLUSIONS.

It must be assumed that some cause is operative in certain cases preventing the healing of defects in the gastric mucosa and is inoperative in others. Even though anhemolytic streptococci are present in practically all gastric ulcers, we cannot convince ourselves that these organisms have been proven as yet to be the factor which either initiates the ulceration or prevents healing. Nevertheless, the constant presence of streptococci in this type of lesion is a suggestive fact and further experiments to determine their significance are being undertaken.

We wish to express our thanks to Dr. A. A. Berg, at whose suggestion this investigation was undertaken. We are further indebted to him for the material studied, for the privilege of reporting abstracts from his clinical records, and for other courtesies extended to us during the progress of this work.

# INDEX TO AUTHORS.


A

**Amoss, Harold L.,** and **Wollstein, Martha.** A method for the rapid preparation of antimeningitis serum, 403
—. See FLEXNER and AMOSS, 683
—. See JACOBS, HEIDELBERGER, and AMOSS, 569
**Auer, J.,** and **Gates, Frederick L.** The absorption of adrenalin after intratracheal injection, 757
— and **Meltzer, S. J.** The intravenous injection of magnesium sulphate for anesthesia in animals, 641
**Avery, Oswald T.** See DOCHEZ and AVERY, 61

B

**Bailey, C. H.** Atheroma and other lesions produced in rabbits by cholesterol feeding, 69
—. The production of amyloid disease and chronic nephritis in rabbits by repeated intravenous injections of living colon bacilli, 773
**Baitsell, George A.** The origin and structure of a fibrous tissue formed in wound healing, 739
**Blake, Francis G.** The etiology of rat-bite fever, 39
**Brown, Wade H.** See PEARCE and BROWN, 443
**Bull, Carroll G.** The influence of typhoid bacilli on the antibodies of normal and immune rabbits, 419
—. See JACOBS, HEIDELBERGER, and BULL, 577
**Burnett, Theodore C.** See ROBERTSON and BURNETT, 631

C

**Celler, Herbert L.,** and **Thalhimer, William.** Bacteriological and experimental studies on gastric ulcer, 791
**Cooke, J. V., Rodenbaugh, F. H.,** and **Whipple, G. H.** Intestinal obstruction. VI. A study of non-coagulable nitrogen of the blood, 717

D

**Dochez, A. R.,** and **Avery, Oswald T.** Antiblastic immunity, 61

E

**Eggstein, A. A.** See PETERSEN, JOBLING, and EGGSTEIN, 491, 499

F

**Flexner, Simon,** and **Amoss, Harold L.** Chemical versus serum treatment of epidemic meningitis, 683
**Futaki, Kenzo, Takaki, Itsuma, Taniguchi, Tenji,** and **Osumi, Shimpachi.** The cause of rat-bite fever, 249

G

**Gates, Frederick L.,** and **Meltzer, S. J.** An experimental study of the additive and antagonistic ac-

tions of sodium oxalate, and salts of magnesium and calcium in the rabbit, 655
—. See AUER and GATES, 757
**Grey, Ernest G.** An experimental study of the effect of cholecystgastrostomy on gastric acidity, 15

H

**Havens, L. C.** See ROSENAU and HAVENS, 461
**Heidelberger, Michael.** See JACOBS, HEIDELBERGER, and AMOSS, 569
—. See JACOBS, HEIDELBERGER, and BULL, 577
**Hoki, Rokuro.** See INADA, IDO, HOKI, KANEKO, and ITO, 377
**Hooper, C. W., and Whipple, G. H.** Icterus. A rapid change of hemoglobin to bile pigment in the pleural and peritoneal cavities, 137
**Hopkins, J. G.** See ZINSSER and HOPKINS, 323
—. See ZINSSER, HOPKINS, and MCBURNEY, 329, 341
**Hull, Thomas, G.** See RETTGER, HULL, and STURGES, 475

I

**Ido, Yutaka.** See INADA, IDO, HOKI, KANEKO, and ITO, 377
**Inada, Ryokichi, Ido, Yutaka, Hoki, Rokuro, Kaneko, Renjiro,** and **Ito, Hiroshi.** The etiology, mode of infection, and specific therapy of Weil's disease (spirochætosis icterohæmorrhagica), 377
**Ingebrigtsen, Ragnvald.** A contribution to the biology of peripheral nerves in transplantation. II. Life of peripheral nerves of mammals in plasma, 251
**Ito, Hiroshi.** See INADA, IDO, HOKI, KANEKO, and ITO, 377
**Ito, Tetsuta,** and **Matsuzaki, Haruichiro.** The pure cultivation of *Spirochæta icterohæmorrhagiæ* (Inada), 557

J

**Jacobs, Walter A.** The bactericidal properties of the quaternary salts of hexamethylenetetramine. I. The problem of the chemotherapy of experimental bacterial infections, 563
—, **Heidelberger, Michael,** and **Amoss, Harold L.** The bactericidal properties of the quaternary salts of hexamethylenetetramine. II. The relation between constitution and bactericidal action in the substituted benzylhexamethylenetetraminium salts, 569
—, —, and **Bull, Carroll G.** The bactericidal properties of the quaternary salts of hexamethylenetetramine. III. The relation between constitution and bactericidal action in the quaternary salts obtained from halogenacetyl compounds, 577
**Jobling, James W.** See PETERSEN, JOBLING, and EGGSTEIN, 491, 499
**Jones, F. S.** A transplantable carcinoma of the guinea pig, 211
—. See ROUS and JONES, 549, 601

K

**Kaneko, Renjiro.** See INADA, IDO, HOKI, KANEKO, and ITO, 377
**Karsner, Howard T.** The pathological effects of atmospheres rich in oxygen, 149
**Kilgore, A. R.** See WHIPPLE, RODENBAUGH, and KILGORE, 123
**King, W. V.** Experiments on the development of malaria parasites in three American species of *Anopheles*, 703

**Kleiner, Israel S.** The disappearance of dextrose from the blood after intravenous injection, 507

**Krumbhaar, Edward B., Musser, John H., Jr.,** and **Peet, Max M.** The relation of the spleen to blood destruction and regeneration and to hemolytic jaundice. XIV. Changes in the blood following diversion of the splenic blood from the liver. A control study of the effects of splenectomy, 87

— and —. The relation of the spleen to blood destruction and regeneration and to hemolytic jaundice. XV. The resistance to hemolytic agents of dogs in which the splenic blood has been diverted from the liver, 97

L

**Larkin, John H.,** and **Levy, I. J.** A pathological study of syphilitic aortitis and its serology, 25

**Levy, I. J.** See LARKIN and LEVY, 25

**Lewis, Dean.** The appearance of the pressor substance in the fetal hypophysis, 677

**Lewis, Paul A.** The distribution of trypan-red to the tissues and vessels of the eye as influenced by congestion and early inflammation, 669

**Loeb, Leo.** See SPAIN and LOEB, 107

M

**McBurney, Malcolm.** See ZINSSER, HOPKINS, and MCBURNEY, 329, 341

**MacNider, William deB.** The inhibition of the toxicity of uranium nitrate by sodium carbonate, and the protection of the kidney acutely nephropathic from uranium from the toxic action of an anesthetic by sodium carbonate, 171

**Mann, Frank C.** A study of the gastric ulcers following removal of the adrenals, 203

**Matsuzaki, Haruichiro.** See ITO and MATSUZAKI, 557

**Meltzer, S. J.** See AUER and MELTZER, 641

—. See GATES and MELTZER, 655

**Musser, John H., Jr.** See KRUMBHAAR and MUSSER, 97

—. See KRUMBHAAR, MUSSER, and PEET, 87

O

**Oliver, Jean.** A further study of the regenerated epithelium in chronic uranium nephritis. An anatomical investigation of its function, 301

**Osumi, Shimpachi.** See FUTAKI, TAKAKI, TANIGUCHI, and OSUMI, 249

P

**Pearce, Louise.** Klossiella infection of the guinea pig, 431

— and **Brown, Wade H.** Chemopathological studies with compounds of arsenic. IV. The character and distribution of renal injury produced by arsenicals as indicated by the processes of repair, 443

**Peet, Max M.** See KRUMBHAAR, MUSSER, and PEET, 87

**Petersen, William, Jobling, James W.,** and **Eggstein, A. A.** Serum changes and the cause of death in experimental pancreatitis. Studies on ferment action. XXX, 491

—, —, and —. Serum changes following thyroparathyroidectomy. Studies on ferment action. XXXI, 499

Q

**Quinby, William C.** The function of the kidney when deprived of its nerves, 535

R

**Rettger, Leo F., Hull, Thomas G.,** and **Sturges, William S.** Feeding experiments with *Bacterium pullorum*. The toxicity of infected eggs, 475

**Robertson, T. Brailsford,** and **Burnett, Theodore C.** The influence of tethelin, and of other alcohol-soluble extractives from the anterior lobe of the pituitary body, upon the growth of carcinomata in rats, 631

**Rodenbaugh, F. H.** See COOKE, RODENBAUGH, and WHIPPLE, 717

—. See WHIPPLE, RODENBAUGH, and KILGORE, 123

**Rosenau, M. J.,** and **Havens, L. C.** Experiments with poliomyelitis in the rabbit, 461

**Rous, Peyton,** and **Jones, F. S.** A method for obtaining suspensions of living cells from the fixed tissues, and for the plating out of individual cells, 549

— and —. The protection of pathogenic microorganisms by living tissue cells, 601

— and **Turner, J. R.** The preservation of living red blood cells *in vitro*. I. Methods of preservation, 219

— and —. The preservation of living red blood cells *in vitro*. II. The transfusion of kept cells, 239

S

**Sattler, Robert Ray.** See WHITE and SATTLER, 613

**Smith, Theobald.** Aberrant intestinal protozoan parasites in the turkey, 293

**Smyth, Henry Field.** The reactions between bacteria and animal tissues under conditions of artificial cultivation. II. Bactericidal action in tissue cultures, 265

—. The reactions between bacteria and animal tissues under conditions of artificial cultivation. III. The action of bacterial vaccines on tissue cultures *in vitro*, 275

—. The reactions between bacteria and animal tissues under conditions of artificial cultivation. IV. The cultivation of tubercle bacilli with animal tissues *in vitro*, 283

**Spain, Kate C.,** and **Loeb, Leo.** A quantitative analysis of the influence of the size of the defect on wound healing in the skin of the guinea pig, 107

**Sturges, William S.** See RETTGER, HULL, and STURGES, 475

T

**Takaki, Itsuma.** See FUTAKI, TAKAKI, TANIGUCHI, and OSUMI, 249

**Taniguchi, Tenji.** See FUTAKI, TAKAKI, TANIGUCHI, and OSUMI, 249

**Thalhimer, William.** See CELLER and THALHIMER, 791

**Torrey, John C.** See WEIL and TORREY, 1

**Turner, J. R.** See ROUS and TURNER, 219, 239

W

**Weil, Richard.** Note on a skin reaction in pneumonia, 11

— and **Torrey, John C.** Immunological studies in pneumonia, 1

**Whipple, G. H., Rodenbaugh, F. H.,** and **Kilgore, A. R.** Intestinal obstruction. V. Proteose intoxication, 123

—. See COOKE, RODENBAUGH, and WHIPPLE, 717
—. See HOOPER and WHIPPLE, 137
**White, Paul Dudley,** and **Sattler, Robert Ray.** The effect of digitalis on the normal human electrocardiogram, with especial reference to A-V conduction, 613
**Wile, Udo J.** Experimental syphilis in the rabbit produced by the brain substance of the living paretic, 199
**Woglom, William H.** Intratesticular implantation of the Flexner-Jobling rat carcinoma, 189
**Wollstein, Martha.** An experimental study of parotitis (mumps), 353
—. See AMOSS and WOLLSTEIN, 403

Z

**Zinsser, Hans,** and **Hopkins, J. G.** Studies on *Treponema pallidum* and syphilis. II. Spirochæticidal antibodies against *Treponema pallidum*, 323
—, —, and **McBurney, Malcolm.** Studies on *Treponema pallidum* and syphilis. III. The individual fluctuations in virulence and comparative virulence of *Treponema pallidum* strains, passed through rabbits, 329
—, —, and —. Studies on *Treponema pallidum* and syphilis. IV. The difference in behavior in immune serum between cultivated non-virulent *Treponema pallidum* and virulent treponemata from lesions, 341

# INDEX TO SUBJECTS.


A

**Acidity:**
Gastric, in relation to cholecystgastrostomy (GREY), 15
**Adrenal:**
Gastric ulcers, following removal of (MANN), 203
**Adrenalin:**
Absorption after intratracheal injection (AUER and GATES), 757
**Amyloid disease:**
*See* Disease.
**Anesthesia:**
Magnesium sulphate (AUER and MELTZER), 641
Sodium carbonate (MACNIDER), 171
**Anopheles:**
Malaria parasites in (KING), 703
**Antiblastic:**
Immunity (DOCHEZ and AVERY), 61
**Antibody:**
Spirochæticidal, against *Treponema pallidum* (ZINSSER and HOPKINS), 323
Typhoid bacilli, influence of, on antibodies (BULL), 419
**Antimeningitis serum:**
*See* Serum, meningitis.
**Aortitis:**
Syphilitic, pathological study of (LARKIN and LEVY), 25
**Arsenic:**
Compounds (PEARCE and BROWN), 443
**Arsenicals:**
Renal injury from (PEARCE and BROWN), 443
**Atheroma:**
Cholesterol feeding producing (BAILEY), 69
**Atmosphere:**
Oxygen in (KARSNER), 149

B

**Bacillus:**
*coli*, injections of (BAILEY), 773
Typhoid. *See* Typhoid.
**Bacteria:**
*pullorum* (RETTGER, HULL, and STURGES), 475
Reactions between animal tissues and (SMYTH), 265, 275, 283
**Bactericidal:**
Action on tissue cultures (SMYTH), 265
Benzylhexamethylenetetraminium salts, bactericidal action of (JACOBS, HEIDELBERGER, and AMOSS), 569
Halogenacetyl compounds, bactericidal action of (JACOBS, HEIDELBERGER, and BULL), 577
Hexamethylenetetramine, bactericidal properties of (JACOBS), 563
(JACOBS, HEIDELBERGER, and AMOSS), 569
(JACOBS, HEIDELBERGER, and BULL), 577
**Benzylhexamethylenetetramine:**
Salts, substituted (JACOBS, HEIDELBERGER, and AMOSS), 569
**Bile:**
Pigment, change of hemoglobin to (HOOPER and WHIPPLE), 137

**Biology:**
Peripheral nerves in transplantation (INGEBRIGTSEN), 251
**Blood:**
Cell, red. *See* Erythrocyte.
Destruction and regeneration in relation to spleen (KRUMBHAAR and MUSSER), 97
(KRUMBHAAR, MUSSER, and PEET), 87
Dextrose in, disappearance of (KLEINER), 507
Nitrogen of (COOKE, RODENBAUGH, and WHIPPLE), 717
Splenic, from liver, changes in the blood following diversion of (KRUMBHAAR, MUSSER, and PEET), 87
—, — —, resistance to hemolytic agents following diversion of (KRUMBHAAR and MUSSER), 97
**Brain:**
Syphilis produced by brain substance (WILE), 199

C

**Calcium:**
Salts, additive and antagonistic action of (GATES and MELTZER), 655
**Carbonate:**
Sodium. *See* Sodium.
**Carcinoma:**
Flexner-Jobling rat (WOGLOM), 189
Pituitary effect on (ROBERTSON and BURNETT), 631
Transplantable (JONES), 211
**Cell:**
Blood, red. *See* Erythrocyte.
Living, suspensions of (ROUS and JONES), 549
Plating (ROUS and JONES), 549
Tissue protecting microorganisms (ROUS and JONES), 601
**Chemical:**
Treatment versus serum treatment of meningitis (FLEXNER and AMOSS), 683
**Chemotherapy:**
Bacterial infections (JACOBS), 563
**Cholecystgastrostomy:**
Acidity, gastric, in relation to (GREY), 15
**Cholesterol:**
Atheroma produced by feeding (BAILEY), 69
**Colon bacillus:**
*See Bacillus coli.*
**Conduction:**
A-V, influenced by digitalis (WHITE and SATTLER), 613
**Cultivation:**
*Spirochæta icterohæmorrhagiæ* (Inada) (ITO and MATSUZAKI), 557
Tissue, action of bacterial vaccines on (SMYTH), 275
—, bactericidal action in (SMYTH), 265
Tubercle bacilli (SMYTH), 283

D

**Destruction:**
Blood, in relation to spleen (KRUMBHAAR and MUSSER), 97
(KRUMBHAAR, MUSSER, and PEET), 87
**Dextrose:**
Disappearance from blood (KLEINER), 507
**Digitalis:**
Effect on normal human electrocardiogram (WHITE and SATTLER), 613
**Disease:**
Amyloid, colon bacilli producing (BAILEY), 773
Weil's (INADA, IDO, HOKI, KANEKO, and ITO), 377

E

**Egg:**
Infected (RETTGER, HULL, and STURGES), 475
**Electrocardiogram:**
Digitalis effect on normal human (WHITE and SATTLER), 613
**Epidemic:**
Meningitis (FLEXNER and AMOSS), 683
**Epithelium:**
Nephritis, regenerated epithelium in (OLIVER), 301
**Erythrocyte:**
Kept, transfusion of (ROUS and TURNER), 239
Living, preservation of, *in vitro* (ROUS and TURNER), 219, 239
**Etiology:**
Rat-bite fever (BLAKE), 39
— — (FUTAKI, TAKAKI, TANIGUCHI, and OSUMI), 249
Weil's disease (INADA, IDO, HOKI, KANEKO, and ITO), 377
**Eye:**
Tissues, trypan-red in (LEWIS, P. A.), 669

F

**Ferment:**
Action (PETERSEN, JOBLING, and EGGSTEIN), 491, 499
**Fever:**
Rat-bite. *See* Rat-bite fever.
**Fibrous:**
Tissue in wound healing (BAITSELL), 739
**Flexner:**
-Jobling rat carcinoma (WOGLOM), 189

G

**Gastric:**
*See* Stomach.
**Growth:**
Carcinomata (ROBERTSON and BURNETT), 631

H

**Halogenacetyl:**
Compounds, salts from (JACOBS, HEIDELBERGER, and BULL), 577
**Healing:**
Wound, fibrous tissue in (BAITSELL), 739
—, influence of size of defect on (SPAIN and LOEB), 107
**Hemoglobin:**
Change of, to bile pigment (HOOPER and WHIPPLE), 137
**Hemolytic:**
Agents, resistance to, in dogs in which splenic blood has been diverted from the liver (KRUMBHAAR and MUSSER), 97
**Hexamethylenetetramine:**
Quaternary salts of (JACOBS), 563
(JACOBS, HEIDELBERGER, and AMOSS), 569
(JACOBS, HEIDELBERGER, and BULL), 577
**Hypophysis:**
Fetal, pressor substance in (LEWIS, D.), 677

I

**Icterus:** (*See also* Jaundice), (HOOPER and WHIPPLE), 137
**Immunity:**
Antiblastic (DOCHEZ and AVERY), 61
**Immunology:**
Pneumonia, immunological studies in (WEIL and TORREY), 1
**Implantation:**
Intratesticular, of rat carcinoma (WOGLOM), 189
**In vitro:**
Preservation of living red blood cells (ROUS and TURNER), 219, 239

**In vitro**—*continued:*
Tissues cultures (SMYTH), 275, 283
**Infection:**
Bacterial, chemotherapy of (JACOBS), 563
Klossiella (PEARCE), 431
Weil's disease, mode of (INADA, IDO, HOKI, KANEKO, and ITO), 377
**Inhibition:**
Toxicity of uranium nitrate (MACNIDER), 171
**Injection:**
Adrenalin absorption after (AUER and GATES), 757
Colon bacilli (BAILEY), 773
Dextrose disappearance from blood after (KLEINER), 507
Magnesium sulphate, for anesthesia (AUER and MELTZER), 641
**Intestine:**
Obstruction of (WHIPPLE, RODENBAUGH, and KILGORE), 123
(COOKE, RODENBAUGH, and WHIPPLE), 717
**Intoxication:**
Proteose (WHIPPLE, RODENBAUGH, and KILGORE), 123

J

**Jaundice:** (*See also* Icterus),
Hemolytic, in relation to blood destruction and regeneration (KRUMBHAAR and MUSSER), 97
(KRUMBHAAR, MUSSER, and PEET), 87
Infectious. *See* Weil's disease.

K

**Kidney:**
Function, without nerves (QUINBY), 535
Injury by arsenicals (PEARCE and BROWN), 443
Nephropathic, uranium, protection of (MACNIDER), 171
**Klossiella:**
Infection (PEARCE), 431

L

**Lesion:**
Cholesterol producing (BAILEY), 69
**Liver:**
Blood changes following diversion of splenic blood from (KRUMBHAAR and MUSSER), 97
(KRUMBHAAR, MUSSER, and PEET), 87

M

**Magnesium:**
Salts, additive and antagonistic action of (GATES and MELTZER), 655
Sulphate, as anesthetic (AUER and MELTZER), 641
**Malaria:**
Parasites, development of (KING), 703
**Meningitis:**
Chemical versus serum treatment of (FLEXNER and AMOSS), 683
**Method:**
Meningitis serum, rapid preparation (AMOSS and WOLLSTEIN), 403
Preservation of living red blood cells *in vitro* (ROUS and TURNER), 219
**Microorganism:**
Pathogenic, protection of (ROUS and JONES), 601
**Mumps:**
*See* Parotitis.

N

**Nephritis:**
Colon bacilli producing (BAILEY), 773
Uranium (OLIVER), 301

**Nerves:**
Kidney deprived of (QUINBY), 535
Peripheral, biology of, in transplantation, in plasma (INGEBRIGTSEN), 251
**Nitrate:**
Uranium, inhibition of toxicity of (MACNIDER), 171
**Nitrogen:**
Non-coagulable, of blood (COOKE, RODENBAUGH, and WHIPPLE), 717

O

**Obstruction:**
Intestinal (COOKE, RODENBAUGH, and WHIPPLE) 717 (WHIPPLE, RODENBAUGH, and KILGORE), 123
**Oxalate:**
Sodium. *See* Sodium.
**Oxygen:**
Atmospheres rich in (KARSNER), 149

P

**Pancreatitis:**
Experimental (PETERSEN, JOBLING, and EGGSTEIN), 491
**Parasite:**
Malaria (KING), 703
Protozoan, intestinal (SMITH), 293
**Paretic:**
Brain substance producing syphilis (WILE), 199
**Parotitis:**
(WOLLSTEIN), 353
**Pathology:**
Syphilitic aortitis (LARKIN and LEVY), 25
**Peritoneum:**
Hemoglobin, change of, to bile pigment in peritoneal cavity (HOOPER and WHIPPLE), 137
**Pigment:**
Bile, change of hemoglobin to (HOOPER and WHIPPLE), 137
**Pituitary:**
Carcinoma, effect of pituitary on (ROBERTSON and BURNETT), 631
**Plasma:**
Peripheral nerves in (INGEBRIGTSEN), 251
**Plating:**
Cell (ROUS and JONES), 549
**Pleura:**
Hemoglobin, change of, to bile pigment in pleural cavity (HOOPER and WHIPPLE), 137
**Pneumonia:**
Immunological studies in (WEIL and TORREY), 1
Skin reaction in (WEIL), 11
**Poliomyelitis:**
Experimental (ROSENAU and HAVENS), 461
**Pressor:**
Substance in hypophysis (LEWIS, D.), 677
**Proteose:**
Intoxication (WHIPPLE, RODENBAUGH, and KILGORE), 123
**Protozoon:**
Parasites, aberrant intestinal protozoan (SMITH), 293

R

**Rat-bite fever:**
Etiology (BLAKE), 39
— (FUTAKI, TAKAKI, TANIGUCHI, and OSUMI), 249
**Reaction:**
Bacteria and animal tissue (SMYTH), 265, 275, 283
Skin, in pneumonia (WEIL), 11
**Red blood cell:**
*See* Erythrocyte.

**Regeneration:**
Blood, in relation to spleen (KRUMBHAAR and MUSSER), 97
(KRUMBHAAR, MUSSER, and PEET), 87
**Renal:**
*See* Kidney.

S

**Salts:**
Benzylhexamethylenetetraminium (JACOBS, HEIDELBERGER, and AMOSS), 569
Magnesium and calcium (GATES and MELTZER), 655
Quaternary, from halogenacetyl compounds (JACOBS, HEIDELBERGER, and BULL), 577
—, of hexamethylenetetramine (JACOBS), 563
(JACOBS, HEIDELBERGER, and AMOSS), 569
(JACOBS, HEIDELBERGER, and BULL), 577
**Serology:**
Syphilitic aortitis (LARKIN and LEVY), 25
**Serum:**
Immune, *Treponema pallidum* in (ZINSSER, HOPKINS, and MCBURNEY), 341
Meningitis, rapid preparation of (AMOSS and WOLLSTEIN), 403
Pancreatitis, serum changes in (PETERSEN, JOBLING, and EGGSTEIN), 491
Thyroparathyroidectomy, serum changes after (PETERSEN, JOBLING, and EGGSTEIN), 499
Treatment versus chemical treatment of meningitis (FLEXNER and AMOSS), 683
**Skin:**
Pneumonia, skin reaction in (WEIL), 11
**Skin**—*continued:*
Wound healing of, influence of size of defect on (SPAIN and LOEB), 107
**Sodium:**
Carbonate, effect on nephropathic kidney (MACNIDER), 171
Oxalate (GATES and MELTZER), 655
**Spirochæta:**
*icterohæmorrhagiæ* (Inada) (ITO and MATSUZAKI), 557
*pallida* (ZINSSER and HOPKINS), 323
(ZINSSER, HOPKINS, and MCBURNEY), 329, 341
—, antibodies against (ZINSSER and HOPKINS), 323
—, immune serum (ZINSSER, HOPKINS, and MCBURNEY), 341
— strains (ZINSSER, HOPKINS, and MCBURNEY), 329
**Spirochæticidal:**
Antibodies against *Treponema pallidum* (ZINSSER and HOPKINS), 323
**Spirochætosis icterohæmorrhagica:**
*See* Weil's disease.
**Spleen:**
Blood destruction and regeneration in relation to (KRUMBHAAR and MUSSER), 97
(KRUMBHAAR, MUSSER, and PEET), 87
**Splenectomy:**
Effects of (KRUMBHAAR, MUSSER, and PEET), 87
**Stomach:**
Cholecystgastrostomy, effect of, on gastric acidity (GREY), 15
Ulcer, gastric (CELLER and THALHIMER), 791
(MANN), 203

**Sulphate:**
Magnesium. *See* Magnesium.
**Syphilis:**
(ZINSSER and HOPKINS), 323
(ZINSSER, HOPKINS, and McBURNEY), 329, 341
Aortitis, syphilitic, pathological study of (LARKIN and LEVY), 25
Experimental (WILE), 199

T

**Tethelin:**
Carcinoma, effect of tethelin on (ROBERTSON and BURNETT), 631
**Thyroparathyroidectomy:**
Serum changes after (PETERSEN, JOBLING, and EGGSTEIN), 499
**Tissue:**
Animal, reactions between bacteria and (SMYTH), 265, 275, 283
Bactericidal action on tissue cultures (SMYTH), 265
Cultures, *in vitro* (SMYTH), 275
Eye, trypan-red in (LEWIS, P. A.), 669
Fibrous, in wound healing (BAITSELL), 739
Fixed, suspensions of living cells from (ROUS and JONES), 549
**Toxicity:**
Eggs, infected (RETTGER, HULL, and STURGES), 475
Inhibition of toxicity of uranium nitrate (MACNIDER), 171
**Transfusion:**
Red cells, kept, transfusion of (ROUS and TURNER), 239
**Transplantation:**
Peripheral nerves (INGEBRIGTSEN), 251
**Treponema:**
*pallidum*. *See Spirochæta pallida*.
Virulent (ZINSSER, HOPKINS, and McBURNEY), 341
**Trypan-red:**
Eye, effect of trypan-red on (LEWIS, P. A.), 669
**Tubercle bacillus:**
Cultivation (SMYTH), 283
**Typhoid:**
Bacillus, influence of, on antibodies (BULL), 419

U

**Ulcer:**
Gastric (CELLER and THALHIMER), 791
(MANN), 203
**Uranium:**
Nephritis (OLIVER), 301
Nitrate, inhibition of toxicity of (MACNIDER), 171
Protection of nephropathic kidney from (MACNIDER), 171

V

**Vaccine:**
Bacterial (SMYTH), 275
**Virulence:**
*Treponema pallidum* (ZINSSER, HOPKINS, and McBURNEY), 329, 341

W

**Weil's disease:**
*See* Disease.
**Wound:**
Healing, fibrous tissue in (BAITSELL), 739
—, influence of size of defect on (SPAIN and LOEB), 107

# PUBLICATIONS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

## THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

*The Journal of Experimental Medicine* is published under the auspices of The Rockefeller Institute for Medical Research and is designed to cover the field of experimental medicine. Information as to contributions and subscriptions is given in full on the other side of this cover.

## THE JOURNAL OF BIOLOGICAL CHEMISTRY

EDITED BY

H. D. DAKIN, New York, N. Y.
E. K. DUNHAM, New York, N. Y.
LAFAYETTE B. MENDEL, New Haven, Conn.
A. N. RICHARDS, Philadelphia, Pa.
DONALD D. VAN SLYKE, New York, N. Y.
CLARENCE J. WEST, New York, N. Y.

*The Journal of Biological Chemistry* is issued monthly, and since its foundation in 1905 twenty-four volumes of approximately 550 pages each have been completed. The subscription price is $3.00 per volume to subscribers in the United States and Canada; $3.25 to those in other countries. The number of volumes issued yearly averages three.

## STUDIES FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

The published results of investigations conducted in the Institute, or under its grants, are republished at irregular intervals in volumes designated *Studies from The Rockefeller Institute for Medical Research.* A small number of the *Studies* are distributed free of charge to libraries, learned societies, and laboratories in which medical research is carried on. Price, $2.00 per volume, payable in advance. A special rate of $1.00 per volume is made to those who subscribe to *The Journal of Experimental Medicine* or *The Journal of Biological Chemistry.*

## MONOGRAPHS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Under the head of *Monographs of The Rockefeller Institute for Medical Research* are published from time to time scientific papers which are so extensive, or require such elaborate illustrations, as to render them unsuitable for current periodical issues. *Monographs* will be sent post paid on application, at $1.00 each, payable in advance by check or money order.

**Monograph No. 1.** (Issued June 30, 1910.)

SIMON FLEXNER and J. W. JOBLING. Studies upon a Transplantable Rat Tumor. Plates 1–16. J. W. JOBLING. The Biology of a Mixed Tumor of the Rat. MAUD L. MENTEN. Experiments on the Influence of Radium Bromide on a Carcinomatous Tumor of the Rat. J. W. JOBLING. Spontaneous Tumors of the Mouse. Plates 17–28. J. W. JOBLING. Transplantation Experiments in *Macacus rhesus* with a Carcinomatous Teratoma from Man.

**Monograph No. 2.** (Issued February 15, 1911.)

WILLIAM TRAVIS HOWARD and OSCAR T. SCHULTZ. Studies in the Biology of Tumor Cells. Plates 1–6.

**Monograph No. 3.** (Issued March 15, 1911.)

BENJAMIN T. TERRY. Chemo-Therapeutic Trypanosome Studies with Special Reference to the Immunity Following Cure.

**Monograph No. 4.** (Issued June 24, 1912.)

FRANCIS W. PEABODY, GEORGE DRAPER, and A. R. DOCHEZ. A Clinical Study of Acute Poliomyelitis. Plates 1–13.

**Monograph No. 5.** (Issued September 27, 1915.)

J. P. SIMONDS. Studies in *Bacillus welchii*, with Special Reference to Classification and to Its Relation to Diarrhea.

**Monograph No. 6.** (Issued January 31, 1916.)

JAMES L. STODDARD and ELLIOTT C. CUTLER. Torula Infection in Man. Plates 1–9.

---

All inquiries, correspondence, and subscriptions should be addressed to the

PUBLICATION DEPARTMENT
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
2419-21 GREENMOUNT AVENUE, BALTIMORE
or 66TH STREET AND AVENUE A, NEW YORK

## CONTENTS

KING, W. V. Experiments on the development of malaria parasites in three American species of *Anopheles*. Plates 98 to 105..... 703

COOKE, J. V., RODENBAUGH, F. H., and WHIPPLE, G. H. Intestinal obstruction. VI. A study of non-coagulable nitrogen of the blood..... 717

BAITSELL, GEORGE A. The origin and structure of a fibrous tissue formed in wound healing. Plates 106 to 109..... 739

AUER, J., and GATES, FREDERICK L. The absorption of adrenalin after intratracheal injection..... 757

BAILEY, C. H. The production of amyloid disease and chronic nephritis in rabbits by repeated intravenous injections of living colon bacilli. Plates 110 to 115..... 773

CELLER, HERBERT L., and THALHIMER, WILLIAM. Bacteriological and experimental studies on gastric ulcer..... 791

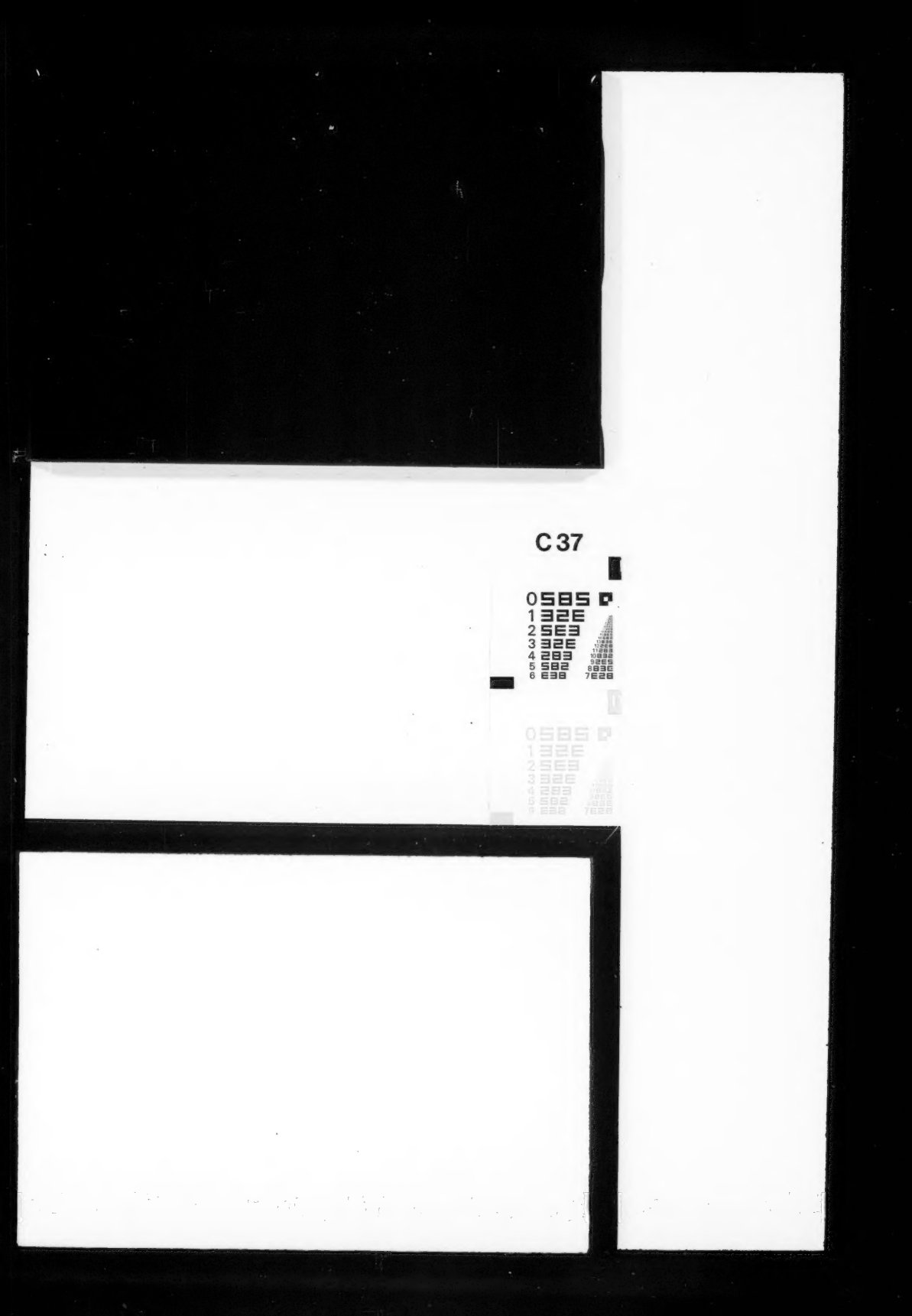
INDEX..... 813

*The Journal of Experimental Medicine* is issued monthly, two volumes appearing in a year. Each volume contains more than 600 pages and many plates and text-figures. Subscriptions will not be accepted to begin in the middle of a volume. Subscribers should state whether they wish their subscription to begin with the January or July volume, unless they wish to leave the decision to the option of the Publishers.

Contributions should be addressed to the Editor of *The Journal of Experimental Medicine*, 66th Street and Avenue A, New York. They should invariably be written in the English language, and limited preferably to twenty printed pages, not counting the space occupied by illustrations. Articles which exceed in length twenty-five printed pages will be returned to the authors in order that their contents may be reduced to this maximum. Authors receive fifty reprints of their papers free of charge; additional copies may be obtained at cost.

Subscriptions (for a year (two volumes), $5.00; single copies, 75 cents) are payable in advance and may be sent to *The Journal of Experimental Medicine*, The Williams & Wilkins Co., Baltimore, Md., or 66th Street and Avenue A, New York. Remittances must be made by draft or check on New York, or by postal money order. Foreign subscriptions may be sent direct to the Publishers, to Gustav Fock, Leipzig, or to Masson et Cie, Paris. If sent to these agents the subscription for a year is 21 marks, 26 francs; for single copies, 3 marks, 4 francs.

For information regarding complete sets and back numbers of *The Journal of Experimental Medicine*, application should be made to the Publication Department of The Rockefeller Institute for Medical Research, 66th Street and Avenue A, New York.


C37